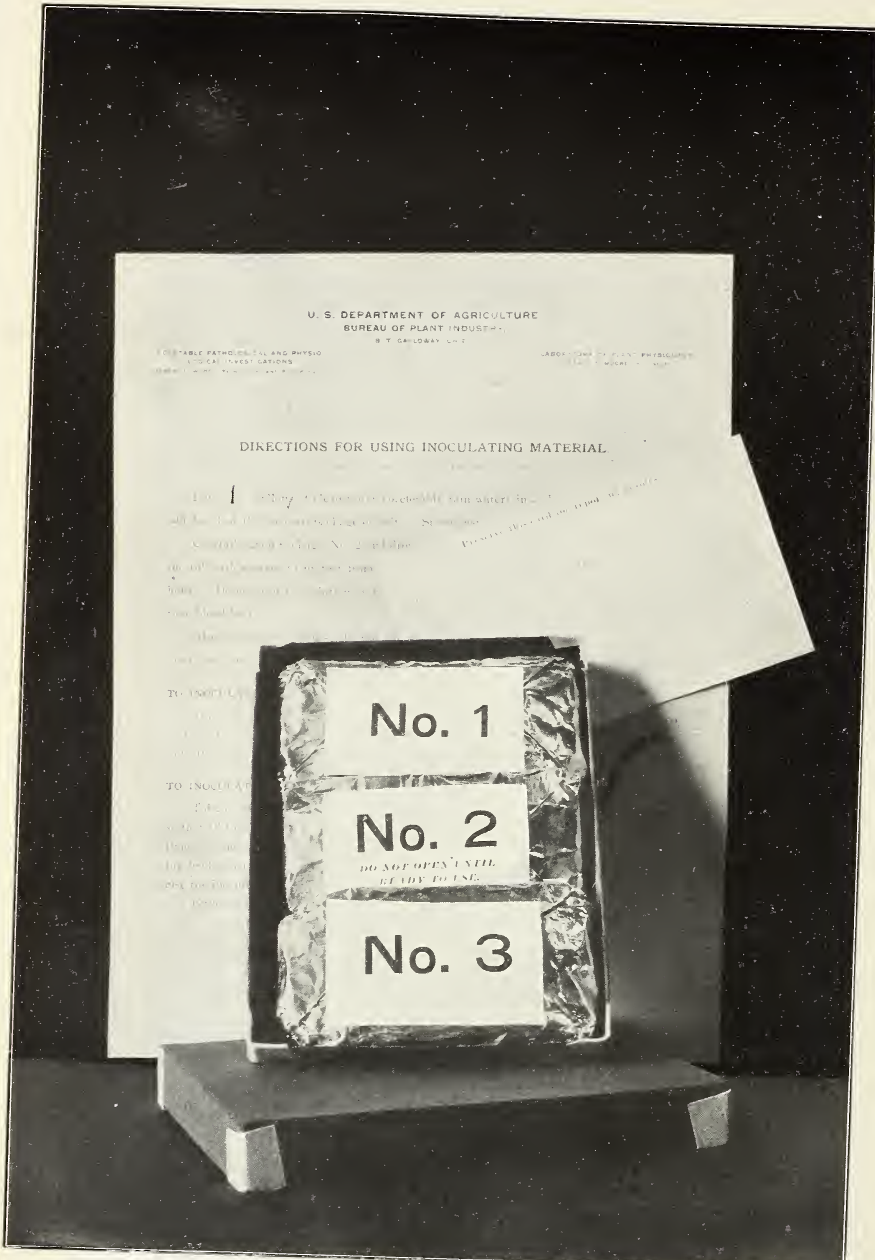

PACKAGE OF INOCULATING MATERIAL SUFFICIENT FOR FOUR ACRES OF ALFALFA.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 71.

B. T. GALLOWAY, *Chief of Bureau.*

SOIL INOCULATION FOR LEGUMES;

WITH

REPORTS UPON THE SUCCESSFUL USE OF ARTIFICIAL CULTURES BY PRACTICAL FARMERS.

BY

GEORGE T. MOORE,

PHYSIOLOGIST IN CHARGE OF LABORATORY OF PLANT PHYSIOLOGY.

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

ISSUED JANUARY 23, 1905.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1905.

BUREAU OF PLANT INDUSTRY.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., November 18, 1904.

Sir: I have the honor to transmit herewith a paper entitled "Soil Inoculation for Legumes; with Reports upon the Successful Use of Artificial Cultures by Practical Farmers," and to recommend that it be published as Bulletin No. 71 of the series of this Bureau. This paper was prepared by Dr. George T. Moore, Physiologist in Charge of the Laboratory of Plant Physiology, in the Office of Vegetable Pathological and Physiological Investigations, and was submitted by the Pathologist and Physiologist with a view to publication.

The subject of nitrogen fixation from the atmosphere is one of the most important problems lying at the foundation of agriculture. The great value of leguminous crops in this connection has long been well known, but until the method of distributing the proper nitrogen-fixing bacteria in pure cultures was perfected by the Department of Agriculture their value in soil improvement was not so great as it now is.

The ten half-tone plates are necessary to a clear understanding of the text.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

The great importance of leguminous crops in maintaining and increasing the fertility of soils was long ago shown to be due to the nitrogen-fixing power of certain bacteria which gain entrance to and live in legume roots. It is now generally recognized that without these bacteria, legumes, like other crops, exhaust the soil of nitrogen. It is thus a matter of the greatest importance in the cultivation of these crops that the proper bacteria be present in the soil under conditions favorable for their development. The old method of inoculating soils by distributing soil from fields containing the desired bacteria is not only expensive, but there is very great danger of spreading at the same time weeds and destructive crop diseases.

Investigators in America, as well as in Europe, appreciate the great importance of securing nitrogen-fixing bacteria in pure cultures for distribution. We had great hope that Doctor Nobbe's nitragin would meet the requirements. These cultures were tested very carefully in this country and in Europe, but were found to be unsatisfactory. We still hoped, however, that the method could be perfected. Mr. W. T. Swingle was therefore instructed to proceed to Europe and confer with Doctor Nobbe regarding the future prospects of his method of pure-culture distribution. Finding that the outlook was rather unsatisfactory, upon Mr. Swingle's return we decided to undertake a thorough investigation of the legume and other nitrogen-fixing organisms, with a view to increasing their agricultural value. The plan was carefully considered and approved by the Chief of the Bureau, the Secretary of Agriculture, and by Congress, and the necessary funds were provided. Finally, we succeeded in securing the services of Dr. George T. Moore to undertake this investigation. With the able assistance of Messrs. Kellerman, Robinson, and Goll, he has succeeded in perfecting the pure-culture method of distribution even beyond our expectations.

Doctor Moore in the course of the investigations soon discovered why it was that the former methods of culture and distribution were so uncertain in their results. He worked out improved methods of making the cultures and increasing by growth in non-nitrogenous media the nitrogen-fixing power of the organisms, and perfected a method of

drying them by which their activity can be preserved indefinitely. These processes have been patented by the Department in the name of Doctor Moore for the purpose of protecting them for the use of the general public.

It is now possible as a result of this work to inoculate at very small expense the seed of all leguminous plants which it may be desirable to cultivate. Bacteria for various legumes were distributed during the past year to a very large number of applicants scattered in nearly every section of this country and in many foreign countries. The results obtained have, as a whole, been extremely satisfactory. The report submitted herewith presents the results of the work up to the present time.

While investigation in connection with legumes is at present the most important phase of the work, careful attention is also being given to nitrogen-fixing bacteria which occur in connection with other plants, and especially those forms which live independently of special plants.

The facts presented in this report demonstrate the great practical value of thorough and accurate scientific investigation of the problems lying at the foundation of agriculture.

ALBERT F. WOODS,
Pathologist and Physiologist.

OFFICE OF VEGETABLE PATHOLOGICAL
AND PHYSIOLOGICAL INVESTIGATIONS,
Washington, D. C., November 17, 1904.

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SOIL INOCULATION FOR LEGUMES;

WITH REPORTS UPON THE SUCCESSFUL USE OF ARTIFICIAL CULTURES BY PRACTICAL FARMERS.

INTRODUCTION.

The primary object in undertaking an investigation of the fixation of nitrogen by the root nodules of legumes was to devise, if possible, some method of bringing about the artificial introduction of the necessary organisms into a soil which was naturally devoid of them, and at the same time to attempt, as far as possible, to correlate and reconcile the vast amount of conflicting evidence that has been accumulated by various investigators in regard to the exact nature of the organism, where the nitrogen is fixed, the effect upon the host, and similar problems.

It will not be possible in an article of this kind to give more than a brief historical sketch of the work that has been done by previous investigators, but in view of the satisfactory review of the literature up to 1892 by Atkinson[a] and by Jacobitz[b] in 1901 an exhaustive consideration of the subject in this way hardly seems necessary.

THE FIXATION OF FREE NITROGEN.

Ever since anything has been known in regard to plant nutrition and the necessary part that various gases and minerals play in the successful growing of crops, scientific men have realized the tremendous importance of conserving the world's store of nitrogen, and have made every effort either to husband or to increase all available sources of supply. In the early days, when it was first being realized that nitrogen was so essential to plant life—in fact, was at the very foundation of agriculture—no particular alarm was felt. Botanists had demonstrated that plants obtained their carbon from the carbon dioxid of the air, and since this gas is present in so much less quantity than nitrogen it was believed that by no possible means could the most

[a] Bot. Gaz., xviii, pp. 157, 226, 257. 1893.

[b] Centralbl. für Bakt., Par. u. Infec., II Abt., VII. November, 1901.

essential of plant foods be exhausted. However, when it was shown that plants were unable to use free nitrogen and must obtain it directly from the soil in a highly organized form, the importance of the problem increased greatly, and the gravest consequences were predicted by those familiar with the rapidity with which this valuable element was being wasted. But a short time ago Sir William Crookes[a] predicted that within thirty or forty years England would experience a wheat famine, due to the exhaustion of nitrogen in the soil, that would be appalling in its effect; and Prof. Bela Korasey's warnings to Hungary have been even more emphatic. Indeed, Liebig, more than fifty years ago, in speaking of one of the most common methods of destroying sources of available nitrogen, said:

> Nothing will more certainly consummate the ruin of England than the scarcity of fertilizers. It means the scarcity of food. It is impossible that such a sinful violation of the divine laws of nature should forever remain unpunished, and the time will probably come for England, sooner than for any other country, when, with all her wealth in gold, iron, and coal, she will be unable to buy the one-thousandth part of the food which she has during hundreds of years thrown recklessly away.

The ways by which combined nitrogen is rendered unavailable for plant food are well known and need no elaborate discussion. The constant cropping of land, combined with our modern sewage system, which prevents the return to the soil of such a large and legitimate nitrogen supply, are sufficient to indicate the extent of this loss without considering the destruction of nitrogenous compounds by the denitrifying bacteria, the burning or exploding of nitrate of soda, and the leaching out of this and other salts which would otherwise be most valuable as fertilizers. These things would not merit so much consideration were it not for the fact that, unfortunately, the world's supply of two of the richest sources of nitrogen—guano and saltpeter—is being exhausted rapidly. Guano has already ceased to be of any great importance, and while it is difficult to obtain precise estimates as to the available amount of saltpeter, it is very certain that at the present rate of its consumption (estimated at 1 billion tons per year) it can not last for a very great length of time, some placing the limit at less than fifty years. It should also be remembered that the natural product, while so rich in nitrogen, is also so expensive that for the general farmer the cost is almost prohibitive. The same may be said of the process recently proposed for the manufacture of nitrogen salts by means of electricity. While the discovery and perfection of such a method is calculated to calm the fears of those who predict a nitrogen famine, it is not one that appeals very strongly to the farmer so long as the price remains where it is.

Regardless of these facts, there are many well-informed men, both at home and abroad, who have always maintained that the possibility

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[a] Brit. Assoc. Ad. Sci., Bristol, 1898. Presidential Address.

of anything approaching a nitrogen famine is so remote as to be unworthy of consideration. In order that this view may be substantiated, it will be necessary to examine into the conditions existing in nature which permit of the restoring of gaseous nitrogen to a combined form.

One of the sources which was formerly supposed to be of considerable importance in returning nitrogen to the earth was by means of the ammonia compounds of nitrous and nitric acid which are present in the air and are often carried into the soil by rainfall. This need not be considered, however, it having been shown that the amount of potassium or sodium nitrate per acre brought into the soil by the rain is, with the exception of a few places in the Tropics, almost infinitesimal, being less than 1 pound per year.

That electricity has some part in fixing nitrogen in a form suitable for plant food has been understood for a considerable length of time. Lightning discharges fix in the soil nitrogen from the air, and a small percentage of this element becomes available in this way. The theory has even been advanced by M. Berthelot that plants in high altitudes will produce good crops without the use of any artificial fertilizer, owing to the greater tension of electricity in these regions, the influence of electric waves permitting the plants to absorb nitrogen in a way that plants not so influenced are unable to do. It certainly seems true that plants elevated to a considerable height will absorb more nitrogen than those at a lower level, but whether this is due to a direct influence of electricity upon the plant itself is perhaps a question.

It has likewise been a matter of common observation that some land allowed to lie fallow frequently increases in its supply of available nitrogen, and to an extent much too great to have been fixed merely by lightning or electrical action of any kind. Consequently, the discovery by Schloesing and Müntz [a] in 1877 that the formation of nitrites from the organic products of animal and vegetable life was produced by living organisms, and the isolation of these bacteria by Jordan and Richards [b] in our own country, as well as by Winogradsky,[c] Frankland,[d] and Warington [e] abroad, were expected to throw much light upon this hitherto little understood subject. Nothing of any practical importance, however, was attempted until after 1891, when Schloesing and Laurent showed that certain organisms had the power of fixing nitrogen in the soil directly from the atmosphere. Experiments were then undertaken along this line, and results obtained which demonstrated that there are unquestionably in the earth a few organisms,

[a] Compt. Rend., Paris, 84: 301.

[b] Mass. State Board of Health Rept., 1890, pp. 865–881.

[c] Ann. de l'Inst. Pasteur, 1890, p. 23.

[d] Phil. Trans. Roy. Soc., London, 1890, B., 107.

[e] Trans. Chem. Soc., 1891, p. 502.

probably both bacteria and algæ, which can directly fix free nitrogen without the aid or interposition of any other plant. Kruger and Schneiderwind[a] have given the result of a test with a bacterium which was able to fix 0.0046 of a gram of nitrogen in 100 cubic centimeters, and numerous other results are recorded showing the beneficial effect of certain organisms of this class upon all crops. Since in many cases the bacteria increased the nitrogen content of the soil so decidedly, it seemed worth while to attempt to bring about an artificial introduction of the peculiar bacteria involved. Before long a patented product, known as alinit, was placed on the market, and numerous trials designed to demonstrate its efficiency were made, but with such indifferent success that the product was withdrawn from sale. Up to the present, therefore, it can safely be said that nothing has been accomplished which would lead to the extensive use of such a process for enriching the land, although the possibility of eventually securing the proper organism and method for distributing it is not unlikely.

BENEFICIAL EFFECT OF LEGUMINOUS CROPS.

From the earliest days of agriculture it has been recognized that all plants belonging to the Leguminosæ had a decidedly beneficial effect upon the soil. Pliny wrote, "The bean ranks first among the legumes. It fertilizes the ground in which it has been sown as well as any manure," and again, "The lupine enriches the soil of a field or vineyard as well as the very best manure. The vetch, too, enriches the soil and requires no attention in its culture." Varro, in De Re Rustica, I, 23, writes, "Legumes should be sown in light soils; indeed, they are planted not so much for their own crop as for the following crop, since when they are cut and kept upon the ground they make the soil better. Thus the lupine is wont to serve as a manure where the soil is rather thin and poor." There are also in ancient writings many other references to the importance and necessity of including some leguminous crop in the regular rotation. Naturally the explanations offered to account for this beneficial effect were various, perhaps the most universal belief being that the root system of these plants was much more extensive than that of grains and root crops and consequently brought up plant food from considerable depths, which not only served the legume, but was likewise available for subsequent crops. Thaer[b] in 1809 advanced the theory that the cultivation of leguminous crops might improve the soil by taking up nutriment from the air and depositing it in the soil through the roots and stubble; but this was merely a conjecture without any experimental basis. Still

[a] Landwirthsch. Jahrb., 19, Heft 4–5, 1900, pp. 801–804.
[b] Rationelle Landwirthsch., 1 Aufl., Bd. 1, 1809.

later, John[a] demonstrated that there was not only an increase in humus after a leguminous crop, but also a definite increase in nitrogen. He was unable, however, to suggest any explanation. In 1854, Boussingault[b] promulgated his classic experiments demonstrating the fact that plants could not assimilate free nitrogen gas. His work was substantiated by the joint labors of Gilbert, Lawes, and Pugh,[c] and although Ville[d] and others still maintained the fallacy of the investigations, it soon became as well established as any fact in plant physiology that the only efficient source of nitrogen supply was in the fixed forms supplied through the roots.

By this time it was beginning to be more generally known that the Leguminosæ were capable of growing in soil practically devoid of nitrogen, and consequently great difficulty was experienced in reconciling what certainly appeared to be two contradictory statements. So well established, however, was the work of Boussingault and others that no very great doubt was cast upon the question of how plants obtained their nitrogen, and an attempt was made to explain the difference through some inherent peculiarity of the legumes themselves. After a series of suppositions, all of which were incorrect, Helriegel[e] announced at a scientific meeting in 1886 that the source of nitrogen for these plants was unquestionably the atmosphere, and two years later, together with Willfarth,[f] he demonstrated the fact that the growth of plants in soil free from nitrogen always occurred after the development of nodules or swellings upon the roots. Later the results of these two men were fully substantiated by many other investigators, and the explanation of the long unsolved problem was made possible. The statement is made by Doctor Young[g] that while the discovery of the fact that the nodules of the legumes enabled them to fix "free nitrogen" is usually ascribed to Helriegel, in reality "Messrs. Hunter and McAlpine were teaching the same fact to their students years before." Attempts to verify this statement have been unsuccessful.

Although Helriegel and Willfarth were probably the first to connect definitely the function of nitrogen fixation with the root nodules, they were not by any means the first who had noticed these structures or had attempted to ascribe some function to them. Malpighi,[h] in 1687,

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[a] Kuhn's Ber. a. d. Lab. d. Landwirthsch. Inst. Halle, 1895, p. 111.

[b] Mem. de Chim. Agric. et de Physiol., Paris, 1854.

[c] Phil. Trans. Roy. Soc., London, 1861.

[d] Compt. Rend., Paris, xxxv, 1852; xxxviii, 1854; xli, 1855.

[e] Tagebl. der 59 Versamml. Deutsch. Naturforscher u. Aerzte in Berlin, 1886.

[f] Verlagsheft zu der Zeitschr. des Vereins für Rübenzuckerindustrie des Deutschen Reichs, Berlin, 1888.

[g] Nineteenth Century, 46, 1899, pp. 782–791.

[h] Opera Omnia, Anatom. Plantar., 1687, Pars Secunda.

gives a description of what he calls the gall formations of the legumes, and in the early part of the last century Karl von Wulffen[a] described the "tiny tubercles" which occur only on legumes, and recommended the cultivation of the white lupine for the improvement of sandy soil. Persoon and Fries, according to Prazmowski,[b] considered the growths to be peculiar fungi related to Sclerotium, and De Candolle[c] believed them to be lenticels. By both Trevisanus[d] and Kolaczek[e] they were regarded as normal plant structures, the first maintaining that they were undeveloped buds with a tubercular formation, while the latter called them "sponge roots," and believed that they served in absorption. The first complete work which gave the detail of the structure of the swellings on legumes was Woronin,[f] who, in 1866, very satisfactorily described them, and, furthermore, for the first time propounded the theory that they were caused by vibrio-like organisms which he discovered within the nodule. From this time up to the date of Helriegel's definite announcement, there were numerous investigators who attempted to solve the problem, but without hitting upon its solution. As late as 1887 Gasparini[g] considered them merely malformed and swollen lateral roots, and the most of those concerned with the question seemed to agree with the view put forward by Eriksson,[h] that the swellings were a purely diseased condition produced by some fungus.

While at first the observations of Helriegel and Willfarth were by no means universally accepted by botanists, the numerous verifications of their work by Lawes and Gilbert,[i] Atwater and Woods,[j] Ward,[k] Kossowitsch,[l] Wagner,[m] and many others soon left no other explanation possible, and it became practically an accepted fact that all legumes were beneficial to the soil because of the presence of peculiar swellings upon their roots which enabled the plants in some way to acquire nitrogen from the air. On the other hand, the nature of the organism which produced the tubercle was not readily decided, and even up to the present time there is considerable discussion as to its character and systematic position.

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[a] Ueber den Anbau der Weissen Lupine im Nördlichen Deutschland, Madgeburg, 1828.

[b] Landwirthsch. Versuchstat., **37**: 169.

[c] Prodromus, II, 1825. Also, Mem. sur la Famille des Legumineuses, 1825.

[d] Bot. Zeitschr., 1853.

[e] Lehrbuch der Botanik, 1856.

[f] Mém. de l'Académie Imp. des Sciences de St. Petersburg, Ser. VII, X, No. 6, 1866.

[g] Ber. der Deutsch. Bot. Gesellsch., 1887.

[h] Acta, Univ. Lund., 1874.

[i] Phil. Trans. Roy. Soc., London, clxxx, B., 1—107.

[j] Amer. Chem. Jour., xiii, 1891.

[k] Phil. Trans. Roy. Soc., London, vol. 178, 1887.

[l] Bot. Zeit., 1892.

[m] Deutsch. Landwirthsch. Presse, 1893.

In addition to the ideas already referred to that the nodules were galls, the result of insect or worm bites, and that they were due to pathogenic fungi, the view was advanced by Brunchorst[a] that the fungus-like strands and the bacteria-like bodies had no connection whatever, the first being of true fungus nature, while the latter were manufactured directly by the plant and after fulfilling an unknown purpose were reabsorbed. This author suggested the name *bacteroids* for the branched and rodlike forms discovered by Woronin, and maintained that the root tubercles were normal structures formed for the purpose of absorbing these when their function was complete. Frank,[b] who had previously advanced other views on the subject, at once accepted the statement of Brunchorst, his former pupil, but went a step further and maintained that even the fungus-like strands were products of the legume cell, and that the whole nodule was merely an organ for absorbing nitrogenous substances from the soil. Schindler[c] likewise coincided with most of these views, and Tschirsch[d] elaborated the point in regard to the origin of the strands and "bacteroids" to the fullest extent.

Enough has been given to show the great diversity of opinion among the leading authorities with regard to both the cause and result of the root nodules of legumes. A great many other observations might be referred to, but they would add so materially to the length of this bulletin and to so little purpose that it seems best not to consider them. The more important investigations necessary for a proper understanding of the present-day opinions will be referred to at the particular point to which they especially apply.

DIRECT EFFECT OF NODULES UPON LEGUMES.

The actual benefit of the presence of root nodules upon various leguminous plants has been so thoroughly demonstrated by numerous observers, both in this country and abroad, that it hardly needs further proof at this time. As has already been referred to, the early work of Helriegel and Willfarth, together with that of Lawes and Gilbert and of Warington in England, and of Atwater and Woods in this country, was quite sufficient to demonstrate the close connection between the fixation of nitrogen in some way by the plant and the presence of the tuber-like swellings on its roots, and there are few, if any, who would maintain that this peculiar function is not, under most circumstances, distinctly beneficial.

The direct effect of the nodule-forming bacteria upon legumes may be demonstrated either by means of greenhouse experiments conducted

[a] Ber. der Deutsch. Bot. Gesellsch., III, 1885.
[b] Deutsch. Landwirthsch. Presse, 1886.
[c] Journ. für Landwirthsch., xxxiii, 1885.
[d] Ber. der Deutsch. Bot. Gesellsch., VII, 1887.

under controlled conditions or by observations upon distinct groups of plants in the field, one lot grown in contact with the bacteria, the other without. Atwater and Woods[a] were the first investigators to show that legumes planted in quartz sand from which all nitrogenous matter had been burned and watered with a nutrient solution devoid of nitrogen in any fixed form would flourish and produce a normal growth when root nodules were present, but would perish as readily as wheat or corn, or similar plants when deprived of the proper bacteria. This experiment has been repeated since by numerous investigators, with various modifications, until it is universally believed that the presence of the bacteria is of the utmost importance and necessity to the legume when growing in a soil containing little or no nitrogen. Indeed, it is possible to demonstrate that a legume growing in a poor sandy soil provided with nodule-forming bacteria will be even more vigorous and produce a better crop than plants growing in moderately rich soil devoid of the bacteria. This fact is well illustrated by the following experiment: Three pots of sand from which all nitrogen had been burned were planted with yellow vetch seed and treated in the following manner: Pot No. 1 was inoculated with nitrogen-fixing organisms and watered with a nutrient solution devoid of nitrogen. Pot No. 2 was not inoculated, but was watered with the same nitrogen-free solution. Pot No. 3 was likewise uninoculated but was supplied with a liberal amount of nitrogen in the form of potassium nitrate. The results were as follows: Pot No. 1, which was inoculated, grew plants averaging 6.16 grams in weight. Pot No. 2, which had no nitrogen provided, showed the poorest growth, average plants but 0.33 gram in weight, while pot No. 3, which was well fertilized, produced plants weighing but 2.65 grams. That is to say, in this particular instance, the inoculated vetch exceeded the uninoculated but fertilized vetch nearly three times in weight, while plants receiving no nitrogen were nearly twenty times smaller than those having nodules. Similar results have been obtained in the field.

Rev. William Brayshaw, of Grayton, Md., reports that he "sowed two lots of seed side by side, one inoculated, the other with 100 pounds of South Carolina rock. Inoculated made double the growth and bade fair to give three times the quantity of hay."

With peas, S. N. Lowry, of Philadelphia, found that "inoculated vines yielded once and a half the crop yielded from ground not inoculated, but which was manured," and Jeremiah Gardner, of Gaffney, S. C., writes, "My peas were better than the peas of others who used commercial fertilizer. They ripened early and evenly. I consider inoculation a boon to agriculture."

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[a] Conn. Storrs Ag. Exp. Sta. Rept., 1889, p. 211; and 1890, p. 312.

H. D. Rixley, of Utica, N. Y., reported that inoculation of five acres of peas was in "every way satisfactory. Got as large a yield per acre as on five acres in same field with heavy barnyard fertilization."

With Canada field peas G. L. Thomas, near Auburn, Me., was able to secure about the same yield with inoculation that he obtained upon similar land after the addition of 800 pounds of fertilizer and 1 ton of barnyard manure, at less than one-half the cost.

One of the first as well as the most satisfactory demonstrations of the beneficial effect of the presence of nodule-forming bacteria upon leguminous crops was made by Prof. J. F. Duggar[a] at the Alabama Experiment Station in 1896 and 1897. In one field where hairy vetch had not been grown previously and the fertilizer used contained phosphoric acid and potash without any nitrogen, the yield was but 235 pounds of hay per acre. On a similar plot, treated in a similar manner, with the exception of the addition of some soil from an old field containing the proper bacteria, the yield of hay was 2,540 pounds, or an increase of over 1,000 per cent. Similar experiments with field peas, clover, and other legumes showed an increase of from 50 to 300 per cent in those plants bearing tubercles as compared with those not possessing them.

In addition to these pioneer experiments of Duggar, there have been numerous other investigators in this country who have obtained similar results. The experiment stations in Mississippi, Kentucky, Kansas, and elsewhere have almost without exception demonstrated most strikingly the immediate advantage of the presence of nodules in all leguminous crops.

Perhaps one of the most satisfactory demonstrations of the ability of legumes to put nitrogen into the soil was the one carried on at the Rothamsted Experiment Station[b] in England for a number of years. Indeed, the great value of the experiments consists in the comparatively long period of time which they cover, thus permitting a thorough comparison of results and a more perfect elimination of external factors. In a series including white clover, vetches, lucern, and other legumes, begun in 1878 and continued for twenty years, it was found that in the first 27 inches of soil the ten sets of samples taken from leguminous plots averaged 6,604 pounds of total nitrogen per acre, while the three sets of wheat soils averaged but 5,847 pounds, showing an average gain of 757 pounds of nitrogen per acre under the influence of leguminous vegetation. It should also be borne in mind that the annual output of nitrogen in the crops from leguminous land was very much greater than from the other plots, in most cases being more than twice the amount of nitrogen per acre. Where an acre of wheat and

[a] Ala. Exp. Sta. Bul. 76, 1897.

[b] U. S. Dept. Agr., Of. Exp. Sta. Bul. 8, 1892.

fallow lands yielded only 12 pounds of nitrogen per annum, white clover produced 24 pounds, so that in addition to the actual accumulation of nitrogen in the soil there is a tremendous output of organic nitrogen in the crops, which has been fixed from the atmosphere, a large part of which will become available if the crop is turned back into the soil as a green manure.

EFFECT OF NODULE-BEARING LEGUMES UPON SUCCEEDING CROPS.

Another graphic way of showing the effect of a leguminous crop possessing nitrogen-fixing nodules upon a soil is to note the vast differences between crops of grain or vegetables that follow legumes and a similar crop grown on fallow land or following a grass or vegetable crop. In addition to the experience of every scientific farmer, which, of course, has given rise to the very common practice of including some legume in rotation, the results of trials by nearly every experiment station in the United States have shown time and again the importance and even necessity of sowing the land to some leguminous crop at the end of a definite period. It is easily proved that part of this benefit is due to the additional amount of nitrogen fixed by the root nodule and not to the unusual length of the root system or other peculiarities of the plant.

J. F. Hickman[a] showed that wheat sown on very poor clay land where *Melilotus alba* had been grown for three years yielded 26.9 bushels per acre, while the same variety on adjoining land which had been in corn and oats produced but 18.6 bushels.

F. E. Emery[b] gives a record of the yields in three and four years from plots on which wheat had been grown continuously. The land upon which a crop of cowpeas was grown every summer increased the yield of grain in 1891 by an average of 13.78 bushels per acre, and in 1892 by 15.6 bushels. In addition, the use of cowpeas as a manure resulted in nearly doubling the number of stalks per stool and increased the height of the plants by nearly 9 inches and the length of the heads by $5\frac{2}{5}$ inches.

F. E. Gardner, and Davenport and Fraser, in the Illinois Experiment Station Bulletins Nos. 37 and 42, show that corn grown in rotation with oats and clover yields 40 per cent more than corn in continuous culture.

A. T. Neale[c] demonstrates that one dollar invested in clover seed returns four times as much as one dollar invested in nitrate of soda. Four acres dressed with pea vines yielded 93 bushels of rye; four acres of timothy sod yielded 18 bushels of rye. Thus, green manure with peas increased the rye crop more than fivefold.

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[a] Ohio Exp. Sta. Bul. 42.
[b] N. C. Exp. Sta. Bul. 91.
[c] Del. Exp. Sta. Rept., 1892.

J. F. Duggar[a] found that oats grown after cowpeas, the vines having been plowed under, produced 10.4 bushels of grain and 229 pounds of straw per acre more than oats grown after German millet plowed under as fertilizer. The average yield of oats per acre after velvet beans was 33.6 bushels, after cowpeas 31.6 bushels, and 8.4 bushels after crab-grass and weeds and German millet.

R. H. Miller and S. H. Brinkley[b] have shown that when crimson clover was plowed under as a manure early in May the yield of potatoes was increased by more than 19 bushels per acre the first year and 34.4 bushels the second year, or more than 50 per cent.

G. B. Irby,[c] in experimenting to determine the value of cowpeas to succeeding crops of cotton, found that fields where no fertilizer was used, but which had been sown to cowpeas, gave a difference of 372 pounds of seed cotton per acre over those where fertilizers had been added.

On the other hand, some experiments with soy beans at the Massachusetts Experiment Station[d] would seem to indicate that legumes do not always have a beneficial effect upon the succeeding crop. W. P. Brooks and H. M. Thompson,[e] in the Massachusetts Hatch Experiment Station Report for 1899, recorded such results. Goesmann[f] some seven years earlier had had the same experience with this crop, finding that the increase in yield was, in general, proportionate to the amount of nitrogen which he had added as fertilizer. In 1896, Goesmann's[g] results showed "that there was not the least evidence of any ability on the part of the soy bean, when grown before a grain crop and harvested, to make nitrogen manuring of the grain crop unnecessary."

The examples demonstrating the great benefit which a leguminous crop has upon the succeeding crop might be extended indefinitely, but enough have been given to prove that it is the almost universal belief, as the result of definite experiments, that a leguminous crop is equal to a considerable amount of nitrogenous fertilizer, and that the crop which follows the legume is benefited to a marked degree. In Germany the number of pounds per acre of nitrogen added to the soil by legumes is estimated at 200 pounds. In the United States the average from sixteen States is 122 pounds. When it is remembered that a high grade of nitrate of soda contains but about 15 per cent of nitrogen, while much that is on the market contains considerably less, it will be seen that a crop of legumes is equal to from 800 to 1,000 pounds of nitrate of soda per acre, which at the present rate for this fertilizer is equal in value to from $20 to $25.

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[a] Ala. Exp. Sta. Bul. 105, 1899.
[b] Md. Exp. Sta. Buls. 31, p. 75, and 38, p. 58.
[c] Ark. Exp. Sta. Bul. 46, 1897, p. 86.
[d] Mass. Hatch. Exp. Sta. Rept., 1899, p. 37.
[e] Ibid., 1899, p. 32.
[f] Ibid., 1892, p. 170.
[g] Ibid., 1896, p. 171.

ARTIFICIAL INOCULATION OF THE SOIL.

Since the desirability of introducing a leguminous crop into rotation seems to be of such importance, and the benefits to be obtained from a nodule-bearing plant are so evident, it is natural that every effort has been made to obtain crops which possess the power of using atmospheric nitrogen. It has been found, however, that although in a great many instances the organisms producing nodules are naturally abundant in the soil and the mere planting of the legume seed is sufficient to produce a crop capable of fixing nitrogen, there are also some localities which are devoid of the necessary bacteria, and in such places the leguminous crop is of no more benefit to the soil than corn or wheat, or other crops whose yield might be a greater source of revenue.

SOIL TRANSFER.

It therefore has become necessary to devise some means of artificially introducing into the soil the nodule-producing bacteria, and naturally the simplest means of accomplishing this has been to transfer earth known to contain the proper organisms and capable of producing nodules to the fields where it was desirable to introduce such bacteria. This soil-inoculation method is one which has been practiced widely, both in this country and abroad, oftentimes with the best results, but not with universal success. Reports have been received from various places stating that even where soil known to contain the proper germs was used the results were not satisfactory. That this failure was not due to the character of the soil or other adverse conditions is proved by the success of other methods of inoculation upon the same kind of land at the same time. The large amount of earth necessary to produce thorough inoculation often makes it a laborious and expensive process when the fields to be treated are at a considerable distance. In addition to the expense and labor involved, however, there is a more serious objection because of the possibility of transferring plant diseases from one field to another.

H. C. Coesten, of Walnut, Kans., reports having transplanted the "leaf-blight" to his field by this method, and many instances are known in the South of the wilt of cowpeas being disseminated by carrying soil from one field to another. There can be no doubt that certain diseases of plants, the spores of which remain in the earth, are widely disseminated by such a means of attempting to produce inoculation by the transfer of soil; and where the disease is one which causes great damage to leguminous crops and is readily transported, it has become necessary to abandon inoculation altogether. There is also great danger of introducing objectionable weeds wherever soil from one locality is introduced into another region. Even though the weeds may not have been serious in the first field, the great numbers of dor-

mant seeds which often require but the slightest change in environment to produce germination are always a menace, and a number of instances have been reported to the Department where the desired leguminous crop was completely choked out by the introduced weed. The director of the Mississippi Experiment Station writes: "Owing to the fact that our alfalfa fields are more or less full of Johnson grass, we are unable to send soil for the purpose of inoculation without distributing this objectionable grass to sections where the farmers are trying to keep it out."

NITRAGIN.

In order to escape the difficulties previously mentioned, Nobbe conceived the idea of bringing about inoculation by means of pure cultures. This was to be accomplished by isolating from the nodule by means of a gelatin plate the right organisms and then transferring to tubes or bottles containing nutrient agar. To this culture of nodule-forming bacteria was given the trade name of "nitragin." Seventeen different kinds of nitragin were prepared from the nodules of as many different plants, and arrangements were made to have them put up on a large scale and placed upon the market by a well-known firm of manufacturing chemists. Experiments with nitragin in Germany met with varying degrees of success. In some instances its use seemed to produce an abundant formation of nodules, while in other cases no benefit could be obtained. In this country the results obtained by Duggar were very satisfactory, but certain other investigators were not able to secure inoculation.

W. M. Munson,[a] while having fair success with soy beans, failed to get any satisfactory results with clovers, peas, vetches, and other legumes, and his results did not warrant the recommendation of the use of nitragin for a leguminous crop.

W. P. Brooks[b] tried nitragin on crimson clover, alfalfa, and common red clover without appreciable effect. B. D. Halstead[c] experimented with a number of legumes and tried three different kinds of nitragin, and as a result there was no evidence that nitragin was of any value in the formation of nodules.

More recently, Maria Dawson,[d] in a series of experiments extending over three consecutive years, concluded that on peat, clay, loam, or ordinary garden soil the inoculation with nitragin proved to be both useless and superfluous.

In spite of these failures, however, a large number of citations might be given which indicate that under certain favorable conditions nitra-

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[a] Maine Exp. Sta. Repts., 1897, p. 144, and 1898, p. 208.
[b] Mass. Hatch Exp. Sta. Rept., 1897, p. 26.
[c] N. J. Exp. Sta. Rept., 1899, p. 375.
[d] Ann. Bot., **15**: 511–519, 1901.

gin was successful in producing nodules upon leguminous crops. The chief difficulties seem to have been in securing cultures of the proper degree of virulence and in preventing deterioration because of being subjected to too much heat or varying degrees of moisture. The age of the culture was also of importance, the manufacturers limiting the time of efficiency to about six weeks. Owing probably to inability to maintain the efficiency of the culture to its highest degree, and the adverse conditions to which it was often subjected during transportation, the percentage of failures in the use of nitragin was so great that its manufacture was given up, and it is no longer for sale under that name. Consequently, even though this preparation had been found to be satisfactory in Europe, the necessity for devising some method of producing nitrogen-fixing nodules free from the objectionable features of transferring soil remained the same. For this reason the Laboratory of Plant Physiology of the Department of Agriculture undertook a scientific investigation of the root-nodule organism, and as a result it is believed that a thoroughly practical and satisfactory method of bringing about artificial inoculation has been devised.

NATURE OF THE ORGANISM.

Before any improvement could be hoped to be made upon methods already in use for bringing about artificial inoculation it was necessary to become thoroughly acquainted with the precise nature of the nodule-forming organism, for, in spite of the fact that these organisms occur in great quantities and that the interior of the nodule constitutes what is practically a pure culture, there has been the widest difference of opinion as to the morphology and life history of these bodies. Beyerinck[a] was the first to cultivate the bacteria successfully, although a year previous Marshall Ward[b] had, by a series of careful experiments, established the fact that the nodule was due to some fungus-like organism present in the soil, and as early as 1886 Woronin[c] expressed a belief that the cause of these abnormal growths was due to foreign organisms, possibly the "vibrio-like bodies" which he was the first to discover and describe.

One reason for the different theories in regard to the true cause of the legume nodule has undoubtedly been on account of the various and diverse forms assumed by the organisms found in the nodule at different times and under different conditions. An examination of a mature nodule of almost any legume will show large numbers of rod-shaped bacteria as well as the characteristic branched forms, but it is probable that in most cases the organism producing the infection is different

[a] Bot. Zeit., xlvi, 1888, pp. 725–804.

[b] Phil. Trans. Roy. Soc., London, vol. 178, 1887.

[c] Mém. de l'Académie Imp. des Sciences de St. Petersburg, Ser. VII, X, No. 6, 1866.

from either of these, being an extremely minute, motile rod usually measuring less than 1 micron in length and about 0.2 of a micron in width. According to Beyerinck, there is a single flagellum attached to the posterior end of these "rovers," but repeated efforts have failed to demonstrate this, although there is no question about motility.

These minute bacteria gain admission to the plant through the root hairs, a number of them often penetrating the same hair. It requires but a short time for them to increase greatly in number, and there is then formed the strandlike colony of bacteria which has been responsible for the idea that the nodules were formed by true fungi. One of the first and most thorough investigations of these fungus-like threads was made by Ward,[a] who followed their development from the root hairs to the cells of the nodule, and came to the conclusion that the bacteria-like bodies originated by a kind of budding from enlarged portions of the "hyphæ." Because of the resemblance of this process to certain known methods of forming spores in the Ustilagineæ, he considered the cause of nodules to be due to a fungus related to this group. Eriksson,[b] Cornu,[c] Prillieux,[d] and Kny[e] have all held similar views as to the fungus origin of the nodule. Frank,[e] while at first adhering to this theory, later came to consider the nodule a natural formation of the legume root developed for the purpose of absorbing nitrogenous substances from the soil. In 1890 the same author[f] returned to the idea of an external cause and accounted for the hyphal-like structures by explaining that they were made up of the protoplasm of the cell and of bacteria-like bodies, to which he gave the name of *Rhizobium leguminosarum*. Some have held the theory that because of the resemblance of these strands to plasmodia, the cause of the nodule must be due to a myxomycete—possibly a form related to the Plasmodiophora of the Crucifereæ.

Careful investigation has demonstrated, however, that these structures resembling hyphæ are in reality nothing more than a zooglœa mass formed by the swelling of the outer layers of the extremely small bacteria which penetrate the root hairs. It is not necessary to assume the presence of minute pores in the cell walls to account for the manner in which the strand passes from one cell to another, as was done by Beyerinck,[a] for the same secretion which enables the original organism to dissolve the wall of the root hair will also in greater quantity produce the same effect upon the root tissue. Although these zooglœa masses, or "infection threads," are usually present in great numbers in the young nodules of most legumes, they do not always occur, and it is not necessary that the bacteria pass from cell to cell

[a] Bot. Zeit., xlvi, 1888, pp. 725–804.
[b] Acta, Univ. Lund., 1874.
[c] Etude sur le Phylloxera, 1878.
[d] Bul. de la Soc. Bot. de France, xxvi, 1879.
[e] Bot. Zeit., 1879.
[f] Landwirthsch. Jahrb., 19, 1900.

in this form. The lupines are particularly free from these strands, it often being difficult to find them even in the root hairs. As the nodule develops, due to the irritation set up in the cells of the root by the entrance of the bacteria, the zooglœa threads, which were at first made up entirely of the minute bacteria, begin to develop bacteria of a larger size which may or may not be motile, according to the conditions under which they are grown. These larger rod-shaped bacteria, measuring from 2 to 5 microns in length and about 1 micron in width, as they become older usually give rise to the peculiar branching forms so frequently described and considered as being peculiar to the legume nodule.

How these branched forms originate has been the cause of some investigation and much speculation. According to Beyerinck,[a] the larger rods have an unsymmetrical, one-sided outline, slightly curved at the middle in such a way that as this swelling increases the two-armed structure is attained. The generally accepted view is that the branched forms are degenerate or involution forms of the rod-shaped bacteria, and for this reason they are frequently designated as bacteroids. Stutzer[b] regards them as a higher rather than a lower type, with which view Hiltner[c] takes issue, he considering them merely enlarged rod bacteria. Greig Smith[d] explains the so-called branching by regarding the nodule organism as a yeast, which, multiplying by budding, causes the various shapes assumed, the capsule often hindering the ready separation. While there is no reason to believe that the nodule-forming organism has any affinities whatever with the yeasts, there are good grounds for the belief that the peculiar, irregular outlines assumed are due to the fact that as a single rod-shaped form divides, it is under certain conditions unable to free itself from the enveloping capsule, and consequently two or more individuals are held together, giving the y or χ appearance. The condition is not so unusual among the bacteria as is generally supposed, similar branching forms occurring in *Mycobacterium denitrificans* and *Pasteuria ramosa*,[e] as well as in the bacillus of tuberculosis.[f]

Further arguments against the degeneration theory of the branched forms are to be found in the fact that they can readily be produced in artificial cultures, provided the conditions are right. A faintly acid medium containing potassium phosphate will usually produce them in a short time, although they are often found upon jelly of different composition. If a solid medium is used, the surface should be covered

[a] Verh. d. Konink. Akad. d. Wetensch. te Amsterdam, **25**: 1887.

[b] Centralbl. für Bakt., Par. u. Infec., II Abt., II, 1896.

[c] Centralbl. für Bakt., Par. u. Infec., II Abt., VI, 273.

[d] Proc. Linnean Soc. of New South Wales, 34, 1899, pp. 653–673.

[e] Ann. de l'Inst. Pasteur, **2**: 165. 1888.

[f] Brit. Assoc. Ad. Sci., 1015–1016. 1896. A. Coppen Jones.

with a thin film of water; if fluid, the amount in the flask must not be too great. Although it is generally supposed that the irregularly branched forms occur only in old nodules, this is by no means the case, as they may frequently be observed in the small, recently formed nodules of young plants.

CROSS-INOCULATION AND SPECIFIC CHARACTERS.

Because of the fact frequently observed that one kind of legume would not produce nodules in soil which abundantly supplied another legume with these growths, it has been supposed that each legume required a special and peculiar nodule organism. Efforts have been made to distinguish between these bacteria specifically, and separate names have been assigned to the microbes from the nodules of peas, beans, clover, etc. Most investigators, however, have been unable to discover any constant difference in the appearance and general characteristics of the bacteria of the various legume nodules, and even Beyerinck,[a] who described at least two distinct groups of these organisms, says that the failure to produce inoculation upon all legumes with one microbe is a difference in variety rather than in species. In order that such an important point might be thoroughly tested, a large number of legumes were grown in pots in the greenhouse for the purpose of testing the efficacy of various cultures derived from nodules of different hosts. The seeds were either planted in quartz sand which had been burned red-hot, or in earth thoroughly sterilized. All of the usual precautions were taken in regard to sterilizing the seed, the water, etc., and the checks proved that these methods were adequate.

It would occupy too much space to give the results of all the cross-inoculation experiments carried on, but a single example will suffice. Nodule-forming bacteria from the common pea (*Pisum sativum*), which had been grown for two weeks upon nitrogen-free media, were used for inoculating seed of the following plants: Crimson clover (*Trifolium incarnatum*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), berseem (*Trifolium alexandrinum*), alsike (*Trifolium hybridum*), sweet clover (*Melilotus alba*), cowpea (*Vigna catjang*), alfalfa (*Medicago sativa*), broad bean (*Vicia faba*), common bean (*Phaseolus vulgaris*), fenugreek (*Trigonella foenum-graecum*), hairy vetch (*Vicia villosa*), scarlet vetch (*Vicia fulgens*), yellow vetch (*Vicia lutea*), blue lupine (*Lupinus angustifolius*), and white lupine (*Lupinus albus*). In every case, with the exception of the lupines, the culture produced nodules. Out of the 25 lupine plants one had four nodules, but this was probably due to insufficient sterilization of the seed. A great many similar cross-inoculations were made in every possible combination, and it was satisfactorily demonstrated

[a] Bot. Zeit., xlvi, 1888.

that it is possible to cause the formation of nodules upon practically all legumes, no matter what was the source of the original organisms, provided they were cultivated for some time upon a synthetic nitrogen-free medium.

It is undoubtedly true that the long adaptation of the bacteria to the special conditions obtaining in a particular species of legume enables such organisms to produce more abundant nodules in a shorter length of time than bacteria isolated from some other legume and grown upon nitrogen-free media. While this is of considerable practical importance, and will probably always make it necessary to distribute the specific organism for the specific crop, it does not in any way indicate that the bacteria found in the nodules of beans, peas, clovers, etc., are separate species. The most that can be maintained is that there is a slight physiological difference due to the long association with a plant of a peculiar reaction which enables the bacteria more easily to penetrate the host upon which they have been accustomed to grow. These slight racial characteristics can readily be broken down by cultivation in the laboratory, and it is entirely possible to secure a universal organism capable of producing a limited number of nodules upon all the legumes which now possess these growths.

As the result of experiments carried on in the Laboratory of Plant Physiology and elsewhere, it is a generally accepted fact at the present time that the organism producing the nodules of the legumes is a single species of bacillus having three well-defined forms. These are: First, a very minute motile rod occurring in the soil and penetrating the root hairs, which may or may not develop peculiar strandlike zooglœa masses; second, a larger rod, measuring from 0.6 of a micron up to 2.5 microns in width and from 1.5 to 5 microns in length. This great diversity in size does not occur in the same culture or nodule, but varies according to different hosts. The larger rods are likewise motile at times, and give rise to the third form, which may appear to be variously branched, but in reality is nothing more than an aggregation of two or more rods held together by a gelatinous sheath. Spores are not known to exist.

Cultivating any of these forms upon gelatin produces slowly developing colonies of a clear, transparent appearance, which do not liquefy the medium. Similar appearing colonies occur upon various solid media without any especial characteristics to distinguish them. The organism is strongly aerobic, growing best at a temperature of from 23° to 25° C., although it may be accustomed to a temperature as high as 40° C.

There does not seem to be any necessity for creating a new group to include these organisms, as has been done by Frank, under the name of Rhizobium, for although there is a certain amount of polymorphism, it is no greater than frequently occurs in the bacteria. Consequently, the name proposed by Beyerinck of *Bacillus radicicola* would

be retained except for the fact that, according to the modern interpretation of this genus, the organism must have flagellæ over the entire surface. According to Beyerinck's own statement and other observations made upon both minute and full-size rods, the flagellæ are found at but one end. For this reason it becomes necessary to transfer the nodule-forming bacteria to the genus Pseudomonas, the name then standing as *Pseudomonas radicicola* (Beyerinck).

METHODS OF CULTIVATION.

The usual method of growing the nodule-forming organism has been to make a medium from a decoction of the particular legume upon which the organism originally grew. This was the method used by Nobbe and Hiltner, and the latter[a] has gone so far as to say that they can only be grown in nutrient media containing legume extract. This, however, is not the case, the number of organic and inorganic substances in both solid and liquid media upon which *Pseudomonas radicicola* will thrive being very great. More than fifty different combinations consisting of various nutrient salts, such as magnesium sulphate, potassium phosphate, ammonium phosphate, together with peptone, sugar, glycerin, asparagin, as well as potato, cabbage, squash, etc., have been found to produce at least a fair growth, although of course an extract of the host plant, plus 1 to 3 per cent peptone, with about 2 per cent cane sugar, will give the most luxuriant growth in the shortest time. As the result of numerous trials, however, it has been found that although the bacteria increase most rapidly upon a medium rich in nitrogen, the resulting growth is usually of very much reduced virulence, and when put into the soil these organisms have lost the ability to break up into the minute forms necessary to penetrate the root hairs. They likewise lose the power of fixing atmospheric nitrogen, which is a property of the nodule-forming bacteria under certain conditions.

For this reason the mere matter of an abundant growth is one of the least desirable considerations in propagating these organisms for any practical purpose, and a medium had to be devised which, while admitting of a fair growth, would at least retain, if not increase, the ability of the organism to produce nodules and fix nitrogen. This condition was met by using an agar for plating out from the nodule to which no nitrogenous salt was added, the usual combination being 1 per cent agar, 1 per cent maltose, 0.1 per cent monobasic potassium phosphate, and 0.02 per cent magnesium sulphate to 100 cubic centimeters of distilled water. While such a medium is not, of course, absolutely devoid of fixed nitrogen, the percentage is so much less than that found in a legume extract-peptone combination that the results are quite satis-

[a] Selskoe Khozyaĭstvo ī Lyesovodstvo, St. Petersburg, 192, pp. 425–462. 1899.

factory. Silica jelly was also used as a solid basis to which the above salts were added, giving a culture medium as free from nitrogen as could be obtained.

Bacteria grown upon media of this character were found to be much more virulent than those cultivated on a rich nitrogenous base, and field experiments by the acre showed the greatest difference in the nodule-producing power of organisms grown by these two methods.[a] That there should be such a considerable increase in the nodule-forming and nitrogen-fixing power of these organisms when grown under different conditions is not surprising when it is remembered how susceptible the bacteria are to a change in their environment and the rapidity with which new generations are formed. Percy Frankland[b] has shown that the mere transfer of the bacillus which ferments calcium citrate from a liquid to a gelatin medium is sufficient to cause it to lose its fermenting power. Rosenau[c] found that a bacterium pathogenic to rats loses its virulence if cultivated in contact with air, and many other instances of the great rapidity with which bacteria may modify their seemingly fixed functions might be given. Therefore, one of the most important advances in developing a method of perfecting a culture of the nodule-forming microbe suitable for practical purposes consisted in getting away from the old and seemingly more natural methods of propagation and resorting to the combination which would result in producing a type of fixed virulence. It would seem that for bacteriology in general, helpful and necessary as the solid nitrogenous media have been, much information of value has been lost by abandoning some of the older and less rapid culture methods.

EFFECT OF VARYING CONDITIONS.

The influence of heat, light, alkalinity, etc., upon the organisms producing nodules is of considerable practical importance, and for this reason a number of experiments were tried to ascertain the effect of various external conditions upon the growth and efficiency of the bacteria.

LIGHT, HEAT, AND AIR.

As the result of numerous tests, it was found that except for the deleterious effect of strong sunlight there seemed to be no difference in organisms grown in the light and in the dark. The optimum temperature is from 23° to 25° C., and above 40° C. there is usually no appreciable growth. It was not possible to produce death by any degree of cold, although below 10° C. practically no multiplication took place. The presence or absence of air was found to be of the utmost importance.

[a] Yearbook of the Department of Agriculture for 1902, pl. xlii, figs. 1 and 2.
[b] Proc. Brit. Inst. Great Brit., **13**: 531. 1890–92.
[c] Bul. V, U. S. Marine Hosp. Serv., 1901.

Cultures from which the air was exhausted soon perished, and even cultures in tubes filled with air but sealed deteriorated rapidly. It is also undoubtedly true that lack of air prevents the formation of the branched forms, which are of the greatest service to the plant in supplying it with nitrogen. This is one reason for certain nodules being of little or no value to the plant, a point which will be discussed more fully in another chapter. The aeration of the medium likewise has considerable to do with increasing the ability of the organism to fix atmospheric nitrogen in liquid cultures, and the necessity for securing an ample supply of air in soil which is to be used for growing legumes can not be too strongly emphasized. An effort was made to determine whether the necessity for a good supply of air was not due to the presence of an abundance of nitrogen gas. Tubes in which the air was replaced by pure nitrogen were able to sustain vigorous cultures of the bacteria for a number of weeks, and it seems probable that this gas is really the only essential obtained from the atmosphere. The action of denitrifying bacteria in the soil, releasing large quantities of nitrogen gas, thus becomes a most important source of supply to nodule-forming organisms.

ACIDS AND ALKALIS.

So far as the growth of the organism upon culture media is concerned, the effect of acids or alkalis within reasonable limits has no decided effect. Experiments tried upon a number of bacteria from various legume nodules proved that it was possible for them to flourish in media containing as high as 0.05 per cent of calcium carbonate, as well as in media containing an equal percentage of free citric and other similar acids. Trials upon plants in pots demonstrated the fact that the bacteria would stand any degree of acidity or alkalinity of the soil that would permit the growth of that particular legume. In general, it may be said that potassium and sodium salts in strengths of from one-third to 1 per cent often entirely inhibit the formation of nodules, and less quantities reduce the formation considerably, while calcium and magnesium salts greatly favor their production. That this action is due to the production of an osmotic state prejudicial to the entrance of the organism through the root hairs, as suggested by Marchal,[a] is a possibility, but the direct effect upon the germs is also a factor which must be considered. On the other hand, there is no question that with lupines and certain other plants adapted to acid soils the addition of calcium and magnesium carbonate is as injurious to the formation of nodules as it is to the plants themselves.

The importance of neutralizing the acidity of certain soils in order to be successful in growing clover, alfalfa, etc., is well known, and

[a] Compt. Rend., Paris, 1901, p. 1032.

the addition of lime is frequently recommended where such crops fail. In such cases it is probable that the acidity of the soil not only is prejudicial to the growth of the plant, but has likewise prevented the development of the nodule-forming bacteria. Thus, the addition of the lime serves a double purpose.

According to Maze,[a] there are but two groups of nodule-forming organisms—those adapted to an acid soil, and normally found on lupine, broom, furze, etc., and those adapted to an alkaline soil, occurring upon most of the common forage and garden legumes. While experience has not borne out this theory in the United States, there can be no doubt about the readiness with which the nodule organism from calcium soils may be accustomed to live upon an acid medium, and the reverse; and there is every reason to suppose that the adaptation of special bacteria to suit special kinds of soil may be readily brought about.

NITRATES.

The fact that the nodules do not occur abundantly upon plants growing in very rich earth has been frequently observed, so that the deleterious effect of nitrogenous substances upon artificial cultures is to be expected. Alkaline nitrates in the proportion of 1 to 10,000 are sufficient to prevent the formation of nodules, and, as has already been referred to, the cultivation of the bacteria upon media containing appreciable quantities of nitrogen for any length of time is sufficient to cause them to lose both the power of infection and that of fixing atmospheric nitrogen. It will thus be seen that many of the factors influencing the size, number, and location of the nodules are those affecting the bacteria quite as much as the plant, and any information in regard to the life history of the organism, together with the physiological effect of conditions and substances with which the nodule-forming bacteria come in contact, will be of much practical importance. Plates II, III, and IV illustrate well the difference in the effect of the same bacteria upon the same kinds of plants in different soils, and fully as striking difference might be shown where the moisture, or the acidity, or the air supply varied.

MOISTURE.

Experiments by Gain[b] and others have shown that with peas, beans, and lupines, watered and unwatered, the number of nodules in moist soil exceeded those in dry soil from ten to twenty times, and experiments in this country have demonstrated most conclusively that the humidity of a soil greatly favors nodule formation. This fact must be due either to the inability of the organism to come in contact with the

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[a] Ann. de l'Inst. Pasteur, xi, 1897, pp. 145–155.
[b] Compt. Rend., Paris, **116**: 1394–1397.

root hairs in the absence of sufficient moisture or to a failure to penetrate the root hairs under such conditions, for drought is in no way fatal to the bacteria.

WHERE IS NITROGEN FIXED?

Having briefly discussed some of the results obtained by the presence of nodules upon the roots of legumes, and having indicated the character of the organisms causing these growths, it is important that we inquire into the precise method by which nodules are of benefit to the plant, if, in fact, they always are beneficial. After it was definitely established that the legumes were actually able to obtain free nitrogen from the atmosphere, naturally the next question was in regard to where and how this gas was fixed. Frank advanced the theory that nitrogen entered the plant just as carbon dioxid does, the transformation into an available form taking place in the leaves in the same way that carbon is obtained. This view soon gave way to a second one, which maintained that the nitrogen was fixed in the soil by the action of the bacteria and then used by the roots in the same way that any combined nitrogen would become available. Still another idea has been that the presence of nodule-forming bacteria in a plant acted as a stimulus which enabled it to use nitrogen gas in some new and unknown manner; and, finally, the explanation has been offered that the nodules with their bacteria act as accumulators of nitrogen which afterwards becomes available for the plant through the destruction of the contents of the nodule. One of the points which might assist in establishing this latter theory would be to demonstrate that the nodule bacteria have the power of combining free nitrogen within their own cells. The chief difficulty in attempting to gain such proof is that it is readily possible that although they possess this function inclosed in the nodule, the power might be lost when removed from contact with the host plant and no fixation would take place under artificial conditions. Indeed, Maze,[a] in discussing the fixation of free nitrogen by the nodule-forming organism, claims that it is acquired in the plant and lost in the soil. That this property is quite unstable in the bacteria of legumes there can be little doubt, and it is not surprising that many investigators have reported an absolute failure in attempting to demonstrate the fixation of nitrogen by these bacteria in pure cultures.

Experiments have shown, however, that the nodule-forming organism in the large rod stage has the property of fixing free nitrogen independent of any host plant, when grown upon the proper media and thoroughly aerated. In order to demonstrate this fact, 90 Ehrlenmeyer flasks containing 100 cubic centimeters each of culture fluid were inoculated with nodule-forming organisms from red clover, soy

[a] Ann. de l'Inst. Pasteur, xi, 1897, pp. 145–155.

bean, white lupine, hairy vetch, berseem, and garden pea. The culture medium contained magnesium sulphate, potassium phosphate, and maltose, and a Kjeldahl determination showed that there was present per 100 cubic centimeters 0.0003 gram of nitrogen as nitrites, making a fluid as free from nitrogen as could be obtained under the circumstances. After inoculation, air which was first passed through a flask filled with pumice stone and sulphuric acid to remove any ammonia was drawn through the flasks by an aspirator. Precautions were also taken to prevent evaporation. Kjeldahl determinations of the inoculated and uninoculated flasks were made at the end of one, two, and three weeks, and in every case a most decided gain in nitrogen was obtained by the end of the third week. Some of the flasks failed to show any difference the first week; in fact, the analysis indicated in a few cases that instead of 0.0003 gram it was impossible to find any trace of nitrogen. This was probably due to the fact that the organism did not develop very rapidly at first and the original amount of combined nitrogen was used before any free nitrogen was fixed. It may be that this took place in all the flasks, but as determinations could not be made oftener than every seven days many of the cultures had begun to gain before the analysis was made.

The actual gain as above determined varied from 0.0002 gram to 0.0022 gram per 100 cubic centimeters. The checks or uninoculated flasks, of which there were twelve, four being analyzed at the end of each week, at no time showed an increase over the original 0.0003 gram per 100 cubic centimeters. Thus, it would seem that there could be little doubt about the power of *Pseudomonas radicicola* to fix free nitrogen independent of any leguminous plant.

A second series of the same number of flasks was started some time after the results from the first analysis were obtained, in order to determine whether or not the nitrogen was combined with the potassium in the medium or was actually contained in the cells of the bacteria. In this set of flasks the fluid medium was varied by adding ammonium phosphate to some and glycerin to others, as well as by substituting cane sugar and peptone for maltose. The results were practically the same as in the first test, except that the percentage of nitrogen fixed was considerably greater in the flasks containing the ammonium phosphate, sometimes showing a gain of 0.0031 gram per 100 cubic centimeters in three weeks. The exact composition of this liquid was as follows: Magnesium sulphate 0.02 gram, potassium phosphate 0.1 gram, ammonium phosphate 1 gram, glycerin 1.5 cubic centimeters, maltose 1 gram, distilled water 1,000 cubic centimeters. After growth had become thoroughly established in these flasks and a Kjeldahl determination showed that nitrogen was being accumulated to a considerable extent, the remainder of the fluid was filtered through

a Pasteur-Chamberland filter for the purpose of removing all the bacteria. The analysis of the filtrate, while showing a small percentage of nitrogen, established without question that a very large proportion of the gain in nitrogen was due to the enormous increase in number of the nodule organisms, each one of which contained a minute quantity of this element.

Since the legume bacteria can fix nitrogen and store it up within themselves, it becomes necessary to investigate carefully the behavior of these organisms within the nodule with a view to determining, if possible, how the nitrogen is supplied to the plant. Analyses of the nodules of legumes show that they frequently contain as high as 7 to 8 per cent of nitrogen, while other parts of the plant will not possess more than 2 per cent. This high percentage is before flowering and the formation of fruit, it being a well-recognized fact that the contents of most of the nodules disappear as the plant reaches maturity and the inclosing tissue shrivels up. Such a high percentage of nitrogen is not constant, however, there being a distinct relation between the character of the nodule and its nitrogen content. As a rule, it may be said that the abnormally large nodules contain the smallest percentage of nitrogen, the most efficient forms being those upon the smaller roots of medium size. Examination of the nodules of such sizes as to be considered unusual shows them to be filled not with branched forms but straight rods, which, as will be seen later, are not suited to supply nitrogen in any quantity. A microscopical examination of the nodule at this time will demonstrate that whereas formerly it was packed full of the branched capsulate organisms, these have now nearly disappeared, leaving only a few rodlike forms. Chemical analysis of the bacteria themselves indicates that they are largely albuminous. Frank found certain nodules developing amylodextrin, and he attempted to distinguish between the organisms forming this substance and those producing albumin. It is not believed, however, that there is any distinction to be made in the contents or substance of the organisms giving rise to the nodule.

The young nodule is at first packed with rod-shaped bacteria and is of a pale red color, changing to greenish gray as the nodule matures and the rods become transformed into the various irregular branched forms so characteristic of these bacteria. Finally, the cells of the roots are able to secrete an enzyme which dissolves the nodule organism when in the branched condition, and by this means renders available considerable quantities of nitrogen, which is then diffused through the plant. This method of absorbing the contents of the nodule is facilitated by the structure of the nodule, which, according to Van Tieghem,[a] originates in the pericycle of the mother root

[a] Bul. de la Soc. Bot. de France, 35: 105–109.

opposite or on each side of the woody bundles. Sometimes the nodules possess from two to four distinct central cylinders, inserted one above the other, at points in the woody bundle of the central cylinder of the mother root. Because of their origin, structure, and disposition, there can be little doubt about nodules being morphologically merely rootlets that have enlarged, the first investigation calculated to establish this fact being made by Van Tieghem[a] and later reaffirmed by Peirce.[b]

NODULES NOT ALWAYS BENEFICIAL.

That the bacteria are almost always able to resist the action of the host plant, except when in the branched condition, is undoubtedly true, although there are a few exceptions in the case of the pea and one or two other plants. If the only source of nitrogen is by dissolving the bacteria, it will readily be seen that should the nodules continue to be filled with the unbranched rods the benefit to the plant will be little or nothing, and the presence of nodules upon the roots may even be a detriment. Too little attention has been paid to this point, the almost universal opinion being that all nodules are able to supply nitrogen to the plant, and any failure in a crop well supplied with these growths must be due to other causes. This is not the fact, however, there being no question that frequently the organisms producing nodules have lost the power of going into the branched condition; and thus, while preventing their destruction by the plant, they defeat the very object for which they are supposed to be so valuable. That this condition is due to the organism itself, and is not the result of lack of vigor on the part of the plant which prevents its secreting the enzyme that will make the bacteria available, is proved by the fact that it is possible to control this situation by modifying the character of the bacteria. Thus, if nodule-forming organisms be grown upon artificial media for a long time, where they are almost invariably in the rod condition, this form becomes so firmly established that plants inoculated with such cultures, although forming nodules, receive practically no benefit, the nodules remaining firm and hard and furnishing no nitrogen to the roots.

It is precisely the same as trying to furnish a plant with its supply of calcium or potassium in an insoluble form. These essentials of plant food may be present, but so long as they remain fixed and will not pass into solution they are valueless to the plant. The nodule organism of most legumes, so long as it retains the rod form, is insoluble, and the plant must be supplied with bacteria capable of passing into the branched stage under the conditions existing in the nodule if

[a] Bul. de la Soc. Bot. de France, 35: 105. 1888.

[b] Proc. Cal. Acad. Sci., 2, June, 1902.

they are to be of service. Thus it is plain that the nitrogen is fixed not by the plant but by the bacteria within its roots, and this element becomes available to the plant only on account of its ability to dissolve and absorb these nitrogen-containing bodies. Consequently, there is after all no conflict with the original dictum of Boussingault that the higher plants can not use directly the nitrogen of the atmosphere. It is no more proper to insist that the legumes themselves can combine nitrogen gas than it is to claim this function for wheat or potatoes. The ability to absorb bacteria rich in nitrogen is the only property peculiar to the nodule-bearing plants. If nematode worms were largely nitrogenous, and violets and other plants infected by them were capable of destroying and absorbing these parasites, it would be just as correct to term the nematode-infected plants "nitrogen-fixing" as it is to ascribe any such function to the legumes.

SYMBIOSIS OR PARASITISM?

Granting the facts just stated, we are at once confronted with the old idea of the supposed symbiotic relation between the bacteria and the plant. Painful as it may be to disturb one of Nature's mutual benefit societies, there seems to be no other way than to consider the nodule-forming bacteria as true parasites which penetrate the roots of the plant for the purpose of obtaining the necessary carbohydrates for food. Fortunately for the host plant, there are certain conditions under which it can overcome the bacteria and eat them up, as it were, thus obtaining a considerable amount of nitrogenous food which would not otherwise have been available. That there is anything ideal or truly symbiotic (in the sense that De Bary used the term) about this arrangement is difficult to comprehend. The only cooperation between bacteria and host seems to consist in the microbe having the best of the situation at first, when it is able to secrete substances injurious to the cells of the legume, and later the host plant retaliates by secreting still other substances which result in the complete destruction of most of the bacteria. So long as it was maintained that the nodule organism could only grow in the root of a legume or upon an extract of these plants, as was claimed by Hiltner[a] and many others, there might have been some slight foundation for the theory, but even this basis is now gone. While not agreeing with Peirce[b] in considering it difficult to understand how the leguminous plant as a whole can benefit by an association with *Pseudomonas radicicola*, which is injurious and finally destructive to the cells in which the bacteria occur, his conclusion regarding the parasitic nature of these bacteria is undoubtedly correct.

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[a] Selskoe Khozyaĭstvo ĭ Lyesovodstvo, St. Petersburg, 1899, pp. 425–462.

[b] Proc. Cal. Acad. Sci., 2, June, 1902.

INFECTION AND FIXATION OF NITROGEN WITHOUT NODULES.

The wide distribution by the Department of Agriculture of cultures for the purpose of experimenting with the artificial inoculation of the soil has led to some very interesting results, some of which may have a considerable bearing upon the final perfection and success of the method now in use. One of the most striking effects reported by some careful observers was the apparent beneficial action of the culture without the formation of nodules. In one instance at a State experiment station three plots of soy beans were planted, one inoculated with a Department of Agriculture artificial culture, another treated with soil from a field which grew nodule-covered soy beans in abundance, and lastly an uninoculated plot for the purpose of checking the other two. The soil for the three experiments was as nearly alike as possible, and the treatment, except as to inoculation, was precisely the same. As the season advanced it was noted that the check plot developed no nodules and rapidly failed; the plot inoculated by the transfer of soil produced nodules and made a fair average growth, but the plot treated with the artificial culture far exceeded it in every way. This was not so surprising until an inspection of the roots showed the entire absence of nodules. No explanation could be offered at the time, but later, when practically the same conditions were noted in some experiments with berseem in the West, plants were secured which threw some light upon the situation. As the result of a careful microscopical examination of the roots, it was found that although no nodules were evident—in fact, did not exist—the cells within the smaller roots were packed with the characteristic branching forms of *Pseudomonas radicicola*, and that undoubtedly the plant was able to obtain considerable benefit from the presence of these organisms.

The same condition has been found in alfalfa, and it is presumed that it was this internal infection which was encountered several years ago in white lupine, although not recognized at the time. Plate V illustrates most strikingly the difference which may occur in plants producing normal nodules and those inoculated but showing no external evidence of infection. The small bunch of alfalfa plants was grown upon rich creek bottom land which had been overflowed and inoculated by the carrying in of bacteria from a melilotus field. These plants were abundantly supplied with nodules. The larger plants were grown upon the same farm, but upon sandy upland where no legumes had been previously planted. Consequently, the seeds were inoculated with a culture supplied by the Department and with most satisfactory results. There was, however, no evidence of nodules, and not until after a microscopical examination of the roots was it known that they were thoroughly infected with nitrogen-fixing bacteria.

Since the production of this peculiar result under control conditions has not as yet been possible, it is difficult to conjecture just what circumstances would produce such an effect. It was undoubtedly of advantage to the plants in all of the known cases and may be a much more universal phenomenon than is supposed. Of course, wherever nodules are produced abundantly there will be little opportunity for detecting internal infection. The absence of nodules in poor soil upon a crop that was failing would seem to indicate that no nitrogen was being fixed and that no bacteria were present. Where legumes have been successful without nodules it has generally been supposed that the soil was rich enough in nitrogen to support the plant and that the requisite bacteria either had never found their way into the soil or because of the excess of nitrogen had been prevented from developing. Cases of this character must be more fully investigated before it is known how frequently inoculation without nodules may occur. That it is not an impossibility is sufficiently evident to warrant further study.

INOCULATION BY PURE CULTURE.

As has already been shown, in order to secure artificially a satisfactory inoculation of any leguminous crop it is necessary that the greatest precaution be taken in procuring the original culture. The method of growing the organism upon some medium relatively free from nitrogen is important in order that its virulence may not be lost, and from the time the bacteria are plated out from the nodule until they are introduced into the soil, every effort must be made to preserve and increase as far as possible the nitrogen-fixing and root-penetrative power of these organisms. Even though the efficiency of the culture be at its highest point, the mere fact of its having to grow for a considerable time under artificial conditions is apt to weaken it; consequently, the means of preserving and distributing the bacteria after they are propagated are fully as important as the method of obtaining them in sufficient quantity for such distribution. This is another reason why the nodule-forming bacteria sent out upon rich nutrient media failed to maintain their original strength, and if it had not been possible to devise some more satisfactory way of delivering these organisms to the farmer, it is probable that but little success could ever have been attained by the pure-culture method. Fortunately, however, although *Pseudomonas radicicola* does not produce spores, the large rods will withstand desiccation for a year or more, and therefore, because they may be sent dry any distance and upon being revived be in the same condition of efficiency with which they started, the problem becomes a very simple one.

The method which has been employed in the Department of Agriculture for the past year has been to saturate absorbent cotton in a

liquid culture of the nodule-forming organism. In this way millions of the bacteria are held within the cotton, and after this is carefully dried out they remain dormant in much the way that seeds do, waiting for the proper conditions to revive them. Where it is possible to obtain sterile utensils and to prevent absolutely the entrance of micro-organisms it is sufficient to insert the inoculated cotton into sterilized water, when in the course of time the bacteria will have multiplied sufficiently to produce a decided clouding of the culture and will be ready to introduce into the ground. This would require too long, however, and it is also difficult, when preparing to treat large quantities of seed, to prevent the entrance of other bacteria, molds, yeasts, etc., all of which may have a deleterious effect upon the growth of the nodule-producing organism. For this reason it has seemed best to prepare the water in such a way as will facilitate the growth of the desired bacteria and yet delay or prevent the development of the forms which might be introduced from the outside. Consequently, two packages of nutrient salts have been distributed with the cotton culture, one containing sugar, magnesium sulphate, and potassium phosphate, and the other ammonium phosphate. (See Pl. I.) By the addition of the first three ingredients to the water containing the cotton saturated with bacteria a solution is formed which is not well adapted for the growth of the organisms usually carried about in the air, but is well suited for the multiplication of the nodule-forming bacteria. The addition of the ammonium phosphate at the end of twenty-four hours tends to increase still further the growth of these bacteria, which are already well started if the temperature has not been too low or too high.

METHODS OF USING LIQUID CULTURE.

After the water containing the nutrient salts and bacteria-laden cotton has been allowed to stand until it becomes milky with the nodule-forming organisms, it is necessary to introduce this culture into the ground. This may be accomplished in two ways, either by moistening the seeds with the fluid, the bacteria adhering to their surfaces and consequently being in close proximity at the time of germination, or by mixing earth or sand with the culture and spreading over the field as one would apply fertilizer. Greenhouse and small-plot experiments indicated no particular advantage of one method over the other, and the hundreds of reports received from all over the country show that either means of introducing the organisms will produce satisfactory results if the directions are properly followed. The sheet of directions which has accompanied each package of inoculating material as distributed by the Department of Agriculture reads as follows:

DIRECTIONS FOR USING INOCULATING MATERIAL.

(Method patented in order to guarantee the privilege of use by the public. Letters Patent No. 755519 granted March 22, 1904.)

Put 1 gallon of clean water (preferably rain water) in a clean tub or bucket and add No. 1 of the inclosed package of salts. Stir occasionally until all is dissolved.

Carefully open package No. 2 and drop the inclosed cotton into the solution. Cover the tub with a paper to protect from dust, and set aside in a warm place for twenty-four hours. Do not heat the solution or you will kill the bacteria—it should never be warmer than blood heat.

After twenty-four hours add the contents of package No. 3. Within twenty hours more the solution will have a cloudy appearance and is ready for use.

To inoculate seed.—Take just enough of the solution to thoroughly moisten the seed. Stir thoroughly so that all the seeds are touched by the solution. Spread out the seeds in a shady place until they are perfectly dry, and plant just as you would untreated seed. If bad weather should prevent planting at once, the inoculated seed, if thoroughly dried, may be kept without deterioration for several weeks. The dry cultures as sent from the laboratory will keep for several months. Do not prepare the liquid culture more than two or three days previous to the time when the seeds are to be treated, as the solution once made up must usually be used at the end of forty-eight hours.

To inoculate soil.—Take enough dry earth so that the solution will merely moisten it. Mix thoroughly, so that all the particles of soil are moistened. Thoroughly mix this earth with four or five times as much, say half a wagonload. Spread this inoculated soil thinly and evenly over the prepared ground exactly as if spreading fertilizer. The inoculated soil should be harrowed in immediately.

Either of the above methods may be used, as may be most convenient.

TIME OF INOCULATION.

The results of numerous laboratory experiments have seemed to demonstrate that it is impossible for the nodule-forming bacteria to penetrate the roots of legumes after they have attained an age of from two to four weeks. Maria Dawson[a] found that plants having roots from 1½ inches to 2 inches long produced no nodules, while those with roots only about one-half inch in length were thoroughly inoculated with the same culture. For this reason it has been considered that it was useless to attempt to add the nitrogen-fixing bacteria to a growing crop, and the directions were adapted to be used at the time of seeding only. Practical experience in the field, however, has given some results which would seem to indicate that under some circumstances the use of inoculating material upon a standing crop of any age will be of benefit.

F. G. Short, of Fort Atkinson, Wis., writes:

> In July the Department sent me a sample of alfalfa bacteria, with directions for application. This was used on a field of alfalfa which had been newly seeded this spring and up to that time had shown a very small growth of yellow, rather stunted plants. I used the bacteria according to directions and can see there is quite a decided change for the better.

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[a] Phil. Trans. Roy. Soc., London, 1899, p. 21.

John C. Lloyd, of Utica, Nebr., used a culture upon 5 acres of alfalfa sown three years ago. The result was "ranker growth than before treatment and much heavier crop of hay. Cut three times and could have cut four, but pastured the last crop."

In Hoard's Dairyman for November 11, 1904, an account is given of the treatment of old alfalfa fields with liquid culture applied by means of a street sprinkler. An experimental trial of this method was made by one of the editors of the paper with "very evident success."

From Levy, Mo., Thomas O. Hudson writes regarding a field of alfalfa planted in 1901 and treated with inoculating material in March, 1904:

> Results good. It was sickly and yellow and spindling, and did not do any good until this year. This year it was dark green and thrifty. I think it will be better next year.

Another report upon an alfalfa field to which bacteria were added during the fourth year was recently sent by U. J. Hess, North Yakima, Wash.:

> The crop, which had been short, pale, and spindling, took on a darker color and a rank growth and yielded, I think, about three times as much as formerly.

The same results have been noted for clover, H. W. Dunlap, Holland Patent, N. Y., reporting that having more of the liquid culture than could be used for some seed he was inoculating, he mixed it with a light loam and spread it upon a part of a field already in clover. The difference in color and size of the plants later on indicated where the soil had been distributed.

Mrs. J. A. Wells, of Bryn Athyn, Pa., tried watering pea vines a month old, with undoubted success, and the results of a similar treatment by John R. Spears, of Northwood, N. Y., are shown on Plate X. Mr. Spears treated his peas with the culture solution with the exception of one row, after they were two or three inches high, and the decided benefit is indicated by his report printed elsewhere in this bulletin.

In the light of these and similar experiments, there can be no doubt that bacteria of a high state of virulence are capable of producing inoculation at practically any time during the life of the legume if the conditions in the soil are favorable. It is probable that similar results have not been previously noted because bacteria of such high efficiency have not been used. While it can not be stated that as satisfactory an inoculation will be obtained in this way as by treating at the time of planting, it certainly seems that under most circumstances where a crop is failing for the lack of nitrogen-fixing bacteria it is worth while making an effort to introduce them, even though the plants be several years old.

WHEN INOCULATION IS UNNECESSARY.

Since the only purpose of the bacteria added to the soil is to furnish nitrogen to the plants in an available form, usually within root nodules, it is evident that where the organisms are already abundant and the crop is thriving, but little benefit can be expected from an additional inoculation. Of course, the nodules may be of the parasitic kind, furnishing little or no nitrogen, or they may be insufficient in quantity, in which case the addition of a fresh lot of bacteria may produce beneficial results. A considerable number of reports have been received, indicating that even with such universally distributed organisms as those occurring on cowpeas and red clover, the artificial inoculation of an old field produced a noticeable increase, and there is every reason to believe that where the land contains bacteria of a less degree of virulence than those sent out in the Department cultures, an inoculation is worth while. On the other hand, it should be remembered that many fields are thoroughly supplied with bacteria of the highest efficiency, and no additional supply, however abundant, will increase the yield.

Inoculation would also be of little, if any, benefit to a rich soil containing a large amount of available nitrogen. As has been shown, the nitrogen-fixing bacteria will not grow well under such conditions, and being in an enfeebled stage the plants are able to withstand their action. Furthermore, the earth already being supplied with a sufficient amount of nitrogen, the plants will draw upon this direct source and produce as abundantly as if provided with nodules. This condition, however, is very undesirable for leguminous crops, and they should not be grown upon such a piece of land unless poorer soil can not be obtained, or unless a legume is the most profitable crop for that region. The use of artificial cultures is preeminently designed for poor soil which it is desirable to bring into condition for producing some root or grain crop demanding large amounts of nitrogen.

WHEN INOCULATION IS NECESSARY.

All legumes grown either for the purpose of enriching the soil or for the crop must, in order to be of the greatest benefit to the land and the plants, be provided with the nitrogen-fixing bacteria. It is believed that the artificial culture is the method most efficient, cheapest, and freest from objectionable qualities. For these reasons inoculation should *always* be practiced under the following conditions:

(1) On poor land which has not previously grown legumes.

(2) On land which, although planted to legumes, has not produced a crop, and the roots of which legumes, upon examination, fail to show the presence of nodules.

It is probable that good results will follow the artificial introduction of bacteria if—

(1) The legumes to be planted belong to another group than those already cultivated upon the land.

(2) The same crop is to be planted upon land which previously produced a yellow and sickly lot of legumes possessing nodules which, instead of being a benefit, acted as parasites.

If the conditions favor the trial, good results *may* be obtained from the use of pure cultures when—

(1) The crop has already been planted and gives evidence of failure due to the absence of bacteria in the soil.

(2) A field which has previously grown good crops of legumes begins to give even a slight evidence that, all other conditions being the same, it is not producing the highest yield. This situation is the hardest to detect, because it depends upon a gradual loss of virulence of the bacteria already in the soil, and the only way of being certain of this condition is to try inoculation and note results.

WHEN TO EXPECT FAILURE WITH INOCULATION.

Failure with inoculation may be expected—

(1) When the directions for preparing the culture media are not carefully followed.

While the method is so simple that anyone observing ordinary care can have no difficulty in securing the proper growth, it is absolutely essential that the few necessary instructions be observed. The fact that thousands of farmers throughout this country with nothing but the printed directions have been able to obtain such satisfactory results is proof that no special knowledge of bacteriology is necessary in preparing and applying the culture fluid. Placing the solution upon ice before it has had time to develop the bacteria, planting the unopened packages in a hole in the ground, pouring the liquid culture into small depressions at intervals of a rod or more, and similar procedures contrary to the directions will not have the effect desired. Unfortunately, the distribution of bacteria for the purpose of increasing the nitrogen supply can not be as definite and complete as if a finished product were sent out. The culture does not itself contain the nitrogen, but simply the organisms which potentially possess the power of fixing nitrogen, and which, if properly handled, will increase in such numbers as to be of material benefit to the plants with which they become associated.

Plate VI will illustrate the difference in results obtained from disregarding directions and from properly following them. Figure 1 represents the best of a very few plants remaining upon a field planted and inoculated more than a year and a half ago. The result was a failure, but because some question was raised as to whether proper

care had been observed in preparing the culture another package of cotton saturated with bacteria from precisely the same lot as the first was sent, with instructions to use especial care in making and applying the solution. The result of the second trial is shown in figure 2, the plants being about two months old.

(2) When the ground is already thoroughly inoculated.

(3) When the soil is so rich in nitrogen as to prevent the growth of nodule-forming bacteria.

(4) When the soil is too acid or too alkaline to permit the development of either plants or bacteria.

(5) When the soil is deficient in other necessary plant foods, such as potash, phosphorus, etc., as well as nitrogen.

It should also be borne in mind that no amount of inoculation will overcome poor results due to bad seed, improper preparation and cultivation of the land, and decidedly adverse climatic conditions.

RESULTS.

All of the foregoing discussion regarding the benefits to be derived from inoculation and the methods devised for propagating and distributing the nitrogen-fixing bacteria amounts to nothing unless it can be shown that these cultures really accomplish, under the general conditions to be found upon any farm, a decided increase in a crop over one grown without inoculation. In order that the bacteria might have the most thorough practical test possible, the Department of Agriculture has for the last year conducted one of the largest experiments of its nature ever undertaken in any country. By the free and unlimited distribution of cultures to practically everyone who was sufficiently interested to request a package, it has been possible to secure about 12,500 tests, under the most varied conditions, in almost every State of the Union. The following list indicates the number of packages distributed up to November 1, 1904.

TABLE I.—*Number of packages of inoculating material (or inoculated seed) distributed from November, 1902, to November, 1904; arranged by States, Territories, and foreign countries.*

State or Territory.	Alfalfa.	Clover.		Pea.			Bean.			Vetch.	Miscellaneous.	Total.
		Red.	Crimson.	Common.	Cowpea.	Field.	Common.	Soy.	Velvet.			
Alabama	242	17	80	10	45	1	7	10	8	109	79	608
Alaska	......	1	......	1	......	......	......	......	....	......	......	2
Arizona	11	......	......	......	......	......	......	......	2	......	1	14
Arkansas	64	15	1	9	28	1	5	2	....	2	10	137
California	171	21	4	61	15	120	41	3	1	30	23	490
Colorado	20	3	......	8	3	1	9	......	....	1	2	47
Connecticut	30	9	......	8	1	......	6	4	....	1	4	63
Delaware	10	2	10	2	3	......	1	......	....	2	3	33
District of Columbia	28	12	6	6	2	......	5	......	2	6	4	71
Florida	40	7	4	22	28	1	28	1	21	17	15	184
Georgia	89	17	26	16	45	5	5	4	3	45	32	287

TABLE I.—*Number of packages of inoculating material (or inoculated seed) distributed from November, 1902, to November, 1904, etc.*—Continued.

State or Territory.	Alfalfa.	Clover.		Pea.			Bean.			Vetch.	Miscellaneous.	Total.
		Red.	Crimson.	Common.	Cowpea.	Field.	Common.	Soy.	Velvet.			
Hawaii	4	1	......	1	2	......	1	2	2	......	......	13
Idaho	16	17	3	4	......	2	4	1	....	4	3	54
Illinois	147	101	3	17	39	3	11	33	1	8	48	411
Indiana	239	103	5	6	31	2	2	16	1	6	36	447
Indian Territory	10	4	......	1	......	......	1	......	....	......	......	16
Iowa	91	41	2	7	6	2	7	9	....	4	32	201
Kansas	122	32	......	7	13	1	11	10	....	2	11	209
Kentucky	66	55	8	13	22	1	5	9	....	8	31	218
Louisiana	50	3	1	6	14	......	1	......	1	3	25	104
Maine	12	21	......	9		......	11	1	....	2	5	61
Maryland	91	28	6	8	13	1	6	7	1	4	9	174
Massachusetts	71	73	3	71	6	4	48	11	....	8	10	305
Michigan	95	95	8	20	9	5	28	10	2	4	26	302
Minnesota	34	38	2	6	......	......	6	1	....	3	5	95
Mississippi	87	3	4	2	5	......	1	1	....	20	19	142
Missouri	211	63	6	10	20	......	8	13	....	7	29	367
Montana	34	10	......	1	......	1	......	......	....	......	4	50
Nebraska	96	19	......	2	4	......	3	3	....	4	6	137
Nevada	3	......	......	......	1	......	......	......	....	......	1	5
New Hampshire	11	22	......	13	2	......	8	2	....	2	8	68
New Jersey	68	32	12	9	7	4	14	2	....	5	3	156
New Mexico	18	2	......	2	......	......	2	......	....	......	......	24
New York	285	174	19	73	25	18	77	30	1	21	31	754
North Carolina	200	54	64	8	48	3	5	11	5	67	38	503
North Dakota	25	16	......	4	......	2	4	......	....	1	4	56
Ohio	273	157	9	20	23	2	24	25	2	10	55	600
Oklahoma	72	2	2	1	12	......	2	4	....	1	4	100
Oregon	126	99	3	23	2	6	15	3	....	61	7	345
Pennsylvania	128	108	15	63	14	3	46	17	....	13	......	407
Philippine Islands	1	......	......	......	......	......	......	......	....	......	......	1
Porto Rico	3	2	......	2	3	......	3	1	2	......	2	18
Rhode Island	6	5	3	3	......	......	4	3	1	1	......	26
South Carolina	70	15	48	8	28	......	5	1	....	35	31	241
South Dakota	28	8	......	5	1	1	3	2	1	1	2	52
Tennessee	185	55	7	17	26	1	10	5	2	9	29	346
Texas	276	10	3	13	28	6	7	2	2	3	77	427
Utah	1	......	......	2	......	......	2	......	....	......	......	5
Vermont	23	21	1	6	1	......	8	2	....	......	2	64
Virginia	541	184	68	26	114	2	7	48	5	56	86	1,137
Washington	106	81	1	27	3	4	24	3	....	22	3	274
West Virginia	34	19	2	5	13	......	3	6	....	2	3	87
Wisconsin	51	45	3	13	8	4	10	11	1	7	7	160
Wyoming	14	......	......	1	......	......	1	......	....	......	......	16
FOREIGN COUNTRIES.												
Australia	2	......	......	1	1	......	......	2	....	1	......	7
British Guiana	1	......	......	......	......	......	......	......	....	......	......	1
Canada	6	6	......	2	......	1	1	1	....	1	......	18
Costa Rica	2	......	......	......	1	......	......	1	1	......	......	5
Cuba	3	......	......	2	5	......	1	2	2	1	1	17
England	2	1	1	......	......	......	3	......	....	2	1	10
France	......	3	......	3	......	......	......	......	....	......	......	6
India	2	......	......	2	3	......	3	......	3	......	......	13
Mexico	3	......	......	......	......	......	......	......	....	......	1	4
South Africa	1	1	1	......	1	......	1	2	....	1	......	8
Unclassified	378	146	79	90	370	8	122	54	13	24	3	1,287
Total	5,129	2,079	523	778	1,094	216	676	391	86	647	871	12,490

REPORTS.

While it has been impossible to receive reports from all experimenters, the percentage of replies has been unusually large and is quite sufficient to enable the formation of a fair opinion as to the value of the cultures distributed. In calling for a report it was, of course, understood that in some cases where the culture was used the result-

ing crop could not be a success, and the users were asked to indicate, as far as possible, when the failure was evidently due to some fault of seed or weather. Likewise, if the soil was shown to have been stocked previously with the proper bacteria and good crops were produced, the use of inoculating material was not expected to be of benefit, and no difference would be detected between treated and untreated land. It is obviously difficult, however, to get all experimenters to make a note of these conditions, a report upon the general result being about all that can be expected in most cases. For this reason the summary of the reports is not as favorable for inoculation as it probably would be if all of the experiments could have been followed in the same way as is possible when such investigations are conducted upon a small scale. It should also be remembered that no selection of the region in which tests were to be made was possible. Experiments with inoculated seed of crops manifestly unadapted for the locality in which they were sown which were reported as a failure of the inoculating material have been recorded as such. In spite of counting unfavorable reports of this kind, which by no fair adjustment should be included, but which, on account of the impossibility of being certain of the conditions, could not be thrown out, the average percentage of failures is less than is generally expected from the indiscriminate planting of seed known to be good.

The tabulated reports so far as received up to November 15, 1904, for all of the principal crops are as follows:

TABLE II.—*Reports of experiments with principal crops.*

	Total reports.	Inoculation resulting in definite increase of crop.	Failures definitely ascribed to bad season, poor seed, weed growth, etc.	No increase in crop; organisms already present in the soil.	No evident advantage from inoculation; nodules not formed.	Percentage of failure. [a]
Alfalfa	1,043	522	287	59	175	25
Red clover	532	302	116	84	30	9
Garden pea	184	102	32	32	18	15
Common bean	174	85	39	23	27	24
Cowpea	290	148	42	68	32	17
Soy bean	129	54	22	11	42	43
Hairy vetch	53	28	13	3	9	24
Crimson clover	49	27	15	4	3	10
Field pea	22	14	4	4		
Velvet bean	10	5	3	1	1	16
Alsike	7	3	1	2	1	25
Sweet pea	7	5		2		
Berseem	2	1			1	
Total	2,502	1,296	574	293	339	26

[a] In computing the percentage of "failures," the number of cases where there resulted no evident advantage from inoculation (fifth column) has been compared with the number which were positive successes (second column), no allowances being made for experiments carried on under conditions precluding any chance of success.

The following reports have been selected with a view to showing the results obtained by using the cultures sent out by the Department of Agriculture upon various crops in practically all of the States where

they can be grown. It is believed that a careful examination of the replies received from so many different experimenters who have had no other instructions than those sent with the inoculating material will demonstrate the success of the new methods devised by the Department in a way that would be impossible by a mere discussion of results obtained in the laboratory or greenhouse.

ALFALFA.

ALABAMA, *Clayhatchie.* E. A. Thompson.—The few plants which were not overcome by the drought show that the inoculation was effectual.

Opelika. Cecil G. Lee.—The nodules formed on the roots, and I have a good stand.

Tuscaloosa. T. J. Ozment.—Seed treated with the bacteria produced 100 per cent more than untreated seed.

ARKANSAS, *Mount Ida.* D. Peters.—Made a crop where it would not grow before. Am of opinion that the inoculation is all right.

CALIFORNIA, *Sanger.* E. C. Southworth.—I had only material enough to inoculate 5 acres; seeded 25 acres to alfalfa. The inoculated seed grew, the other did not. I used it with such success last winter that I feel that I must have it for all this winter's seeding, and am anxious to secure enough for about 900 pounds of alfalfa seed.

Mecca. E. Brauckman.—I am glad to state that the inoculation of the alfalfa field is a success. The soil is, or was, exceedingly salty, growing nothing but saltwort and bushy samphire. I had grave fears whether the microbes could flourish in such soil.

Mesa Grande. Morgan R. Watkins.—The crops (alfalfa and cowpeas) were far in excess of the best results heretofore, though planted on the poorest soil and in the driest weather. Other plantings were absolute failures.

CONNECTICUT, *Granby.* Daniel P. Cooley.—The nodule formation is perfect. No crop has been harvested this season. Has been cut four times to kill weeds. The stand at this date (October 20, 1904) is good.

Marbledale. J. E. Watson.—No alfalfa has ever before grown for me except in a well-prepared seed bed. I have a good stand of alfalfa where I sowed it; have cut it twice and will do so again later in the season. Am forced to believe in its value [i. e., of the inoculating material].

DELAWARE, *Townsend.* James Flanagan.—A heavy storm just as plants came up covered many of them, but those remaining looked nicely and have bacteria nodules on the roots.

IDAHO, *Freese.* I. E. Lobaugh.—Made fine growth for first year. Good stand. Clipped three times. Left on ground.

ILLINOIS, *Hillsboro.* Thomas S. Evans.—The field of alfalfa is perfect; whole field deep rich green, not a single pale or yellow plant. If it does not winterkill it will be the first successful attempt in growing this legume in this section, which success is without doubt due to your inoculating material.

Mount Carmel. W. F. Chipman.—Nodule formation on almost every plant examined yesterday, and foliage rich dark green. Fine, vigorous stand. I find more nodules on the alfalfa sown about a month ago than on that sown last May. The reason perhaps is the difference in the amount of ground covered with each package, the first being about 9 acres, while the last was spread over but 1 acre.

Illinois—Continued.

Mount Morris. D. E. Brubaker.—I find nodules quite evenly distributed over the entire plot of 1 acre. Am much pleased with the success. I saturated seed instead of soil. Three cheers for the discovery.

Mount Morris. A. W. Brayton.—A portion of the field is like the larger plant sent you; apparently the inoculation did good work here. (See Plate VIII.)

Indiana, *Aurora.* E. L. Cannon.—I have got a fine stand. I sowed 300 pounds lime per acre on the clay land; then harrowed. I drilled 3 pecks of oats per acre. I cut 40 bushels oats per acre. Then we had a severe drought. The alfalfa died down, but as soon as we had rain it came up from the roots. It is fine. I got a good crop of oats and a good stand of alfalfa. I have a near neighbor who sowed oats with his alfalfa, mowed them, and left them on the ground for a mulch. He has not nearly as good a stand as I have. He did not inoculate.

Butler. L. G. Higley.—Bacteria for alfalfa was received in good condition about May 1. I prepared it and mixed it with about 20 bushels of rich soil and sowed it on the field after plowing it. I sowed my alfalfa seed May 11, along with 2 bushels of smooth barley per acre. It has done better than any alfalfa that I ever sowed. It stands over a foot high nearly all over the field. There is hardly a square foot of land in the field that is not well set with plants. I took a spade to-day and went in the field to see if I could find any trace of the bacteria, and I soon found that the soil was full of it, every plant having lots of the nodules on the roots. I then went to a field of 2-year-old alfalfa, which never was treated with bacteria, to see if there were any nodules there, and after hunting a long time I found a few very small nodules, but hardly enough to be really worth mentioning. This field is failing and I will have to plow it up. Alfalfa will grow on real rich soil without its bacteria, but I believe it will grow better with it; and if the land is the least bit poor it will starve to death if it has not its bacteria.

Indian Territory, *Pecasset.* Don Nolian.—We had tried it (alfalfa) twice before, this being the third trial. Had perfect stand and have cut three crops and have good covering for winter. There is about 1 acre in lot, and I have taken about 3 tons of hay from it, or a ton at each cutting.

Iowa, *Algona.* Judge W. B. Quarton.—I took one gallon of nice rain water and followed the directions received from your Department with the culture of bacteria, and received the identical results that your Department said I would. I personally inoculated this bushel of seed, then spread it out to dry, took it to the farm the next morning, and planted the seed. * * * I have been upon my farm many times between the middle of July and this writing, taking my pocketknife and digging down to the roots of the alfalfa plant. I have never failed to find plenty of thrifty looking tubercles on the roots, they ranging from one to clusters of one hundred, and I am satisfied that my field is thoroughly and completely inoculated, and I believe that your method is a complete success. * * * I feel like congratulating your Department upon the very thorough and practical work that you are doing in the line of plant industry and especially as to leguminous plants. I hope that you will continue it, because the legume is the one plant, above all others, that fertilizes the soil and at the same time furnishes the protein necessary to balance the food ration in our corn-growing States like Iowa.

Garwin. William S. Dobson.—Good. Seed came up and grew. Had tried same field in alfalfa the year before, but did no good. Has proved inoculation a success. Many have tried alfalfa experimentally in central Iowa, but with indifferent success.

IOWA—Continued.

Hornick. George O. Shedd.—The stand is good. I thought that it would die on the thin, poor, yellow places, but looks now as though it may not. Treated 1 bushel, sowed 4 acres.

KANSAS, *Arkansas City.* Rufus R. Marsh.—The seed was used on sandy knolls in field, and made better showing than balance of field without inoculation.

Halstead. G. R. McWilliams.—The inoculated seed has a good colored plant; the uninoculated plants were yellow and did not make any hay. I would not try alfalfa without inoculating the seed.

Halstead. A. Murray.—The alfalfa inoculated could not have done better. I will not plant any after this without inoculation. I think inoculated alfalfa is as good at 1 year old as uninoculated is at 3 years old.

Holton. R. J. Linscott.—Harvested three crops of hay. An exceedingly thick, even stand. Plenty of bacteria on roots. A success in every way. Hope I can treat alfalfa seed this way every time I sow it, as I never had a successful stand before.

Holton. S. K. Linscott.—All that could be desired. At seven weeks from date of planting it was 10 inches high in some places. Every root so far examined has from one to six nodules. Many thanks for your kindness.

Stockton. J. J. Coppersmith.—Proved very satisfactory. Did about one-fifth better than that not treated, but other chances equal. Amount, 3½ acres; first cutting, 3 tons; second, 5 tons.

KENTUCKY, *Berlin.* John A. Buser.—One acre was planted; one half was inoculated, the other half was not. Received good stand in all parts. On examination of some roots the treated plants had root nodules and the untreated were barren.

Eminence. R. R. Geltner.—I succeeded in getting an excellent stand of alfalfa. Former trials proved a failure where not inoculated. Believe the inoculation will be a great success.

Moreland. N. J. Cone.—Cut alfalfa first year. I inoculated 1 bushel of alfalfa seed and sowed one-half bushel without inoculating. Got very fine stand of that inoculated, not so good on that which was not.

MAINE, *Seal Harbor.* Ida M. Bodman.—The inoculated seed produced a good though not luxuriant crop; the uninoculated seed (or, rather, piece of ground) was more buckwheat weed than alfalfa. My soil is poor, thin, and shallow.

Wayne. S. H. J. Berry.—Last year I tried to raise alfalfa but was unable to get a stand, but this year, by the use of the inoculation, I have a very pretty plot of this valuable grass. I believe it to be what my land requires.

MARYLAND, *McDaniel.* William Bielefeldt.—Inclosed please find your card filled out as to general results. I did not harvest any hay off the field, but pastured it lately. I am sorry that I am not able to give you any definite figures on the crop, and as your card is not large enough to express my appreciation and enthusiasm for your method of inoculation you will please excuse this letter, in which I will try to sum up my observations of the experiment in the following: I inoculated 1,800 pounds of alfalfa seed with the material received. I dried the seed well after inoculating and sowed it from May 1 to August 15. The land is a medium heavy fine clay soil and originally, I think, a fairly good soil, but has been entirely farmed to death with continuous tobacco raising, and after that wouldn't grow any more they followed it up with wheat and corn till that failed to grow any more; then the farm was sold. So I can say the soil is in a very poor condition chemically and physically, so much so that on 2 acres sown with seed not inoculated, alfalfa failed to make a stand at all. But on all ground in the same condition the inoculated seed made a brilliant

MARYLAND—Continued.

stand and is looking a real deep green in color, when nearly everything else is dried up, as we have had no rain for six or seven weeks. In all, allow me to say that in my opinion your Bureau has made the greatest discovery toward helping the growing of alfalfa that could be made, and that you may well be proud of it, and I thank you for giving me a chance to use it. A neighbor adjoining me sowed uninoculated seed three successive times on the same piece of ground and failed to get a stand; that is positive proof of the inoculation being a benefit.

MICHIGAN, *Croton*. E. L. Hornbeck.—I have a nice stand of alfalfa. I think I never before saw young plants push forward as rapidly on the start. The land is sandy but I have a nice young meadow as the results of my applying the bacteria. It was a complete success.

Kiffie. A. L. Rockwell.—Mixed bacteria with soil; sowed broadcast after seeding; harrowed lightly. Seed all grew and made a good stand. Other seed without bacteria failed.

MISSOURI, *Alexandria*. Jasper Blines.—The material is a success. After six weeks I found large bacteria tubercles upon the young alfalfa roots.

Brewer. L. S. Hogan.—Seed sown has plenty of nodules on the roots and promises a good crop next season. The same piece of land sowed to alfalfa in 1902, seed not inoculated, all died out the following summer.

Levy. Thomas O. Hudson.—Planted in 1901. Inoculation good. Alfalfa was sickly and yellow and spindling, and did not do any good till this year after inoculation. This year it has been dark green and thrifty, and I think it will be better next year.

NEBRASKA, *Agee*. Sam Nelson.—Got a good stand and it has made good aftergrowth where twice before it was practically a failure. Am satisfied inoculation gave good results.

Atkinson. H. E. Henderson.—I got a good stand where I had failed twice before. I think it the only safe and sure way to secure a stand.

Liberty. Harry D. Huyck.—I sowed seed and inoculated soil on a 2-acre field of 2-year-old alfalfa to thicken stand. Produced 10 loads of hay as against 5 loads in 1903. All young plants thriving, old plants much better stand, probably due to severe harrowing and inoculation.

Omaha. A. L. Cottrell.—Alfalfa bacteria successful for alfalfa inoculation. Growth larger. Full report of experiment is given in my post-graduate thesis on "Alfalfa as a forage crop for Iowa."

Page. L. M. Butler.—The seed inoculated was more satisfactory in stand and growth than that sown without. Think it is all right.

Utica. John C. Lloyd.—Ranker growth than before treatment and a much heavier crop of hay. Cut three times and could have cut four, but pastured the last crop. The bacteria were used on 5 acres of alfalfa sown three years ago, with above result.

NEW JERSEY, *Rosenhayn*. A. F. Lewis.—This year I have a fine stand on the same ground that I failed on twice without inoculation.

Vineland. E. L. Bolles.—First cutting on May 25, 1904, of 2 to 3 tons from 1 acre (seeded August 25, 1903), nine months from seeding. Scores of trials without inoculation have been made in this section with universal failure. Alfalfa wintered well, while we had a killing winter for crimson clover.

NEW MEXICO, *Nogal*. Ed. C. Pfingsten.—Inoculation applied on seed, no bacteria; soil inoculated shows bacteria on all roots examined. Soil inoculated plants from 20 to 30 inches high, others 6 inches.

New York, *Fillmore.* C. V. Mills.—It looks very promising to go into winter. It had a good color and never turned yellow during its growth. I can find plenty of nodules. I think the bacteria a benefit. Tried growing it two seasons without, and it made a sickly growth.

Amsterdam. Barlow W. Dunlap.—Sowed April 27, after treating seed and drying, 90 pounds to 3½ acres with 1 bushel of barley per acre. Have cut twice, and now have a very thick even stand of alfalfa about 10 inches high, of a very dark-green color. I have recently examined the plants in all parts of the field and find nodules on nearly every root. The same piece was sown with untreated alfalfa seed in 1902. The plants started well, but nearly all died before fall. I could not then find a single nodule on the roots.

Apalachin. C. L. Yates.—Sowed last year on piece adjoining and had no luck. Plowed the ground this year 1st of May and have got a good stand. Think the inoculation was a good thing.

Briarcliff Manor. Walter W. Law, manager, Briarcliff farms.—Good stand. I never could get any to take before.

Canastota. W. R. Groat.—A grand success. Carefully carried out your instructions in preparing the culture and in inoculating the seed; rolled it in and have a heavy even stand 8 inches high over the entire 3¼ acres. Have spent a great deal of time in getting information about what success others have had in these parts, and all complain of not being able to get a good stand, and some sow 1 bushel per acre and then do not get it. This is my first attempt, but I am satisfied that my success is due to the inoculation, but one in preparing must carefully carry out your instructions to insure success.

Stanfordville. Albert Knapp.—This seed was sown on ground where I tried to get alfalfa the year before and failed, the plants turning yellow when about six weeks grown, and dying. I now have a fine stand on same ground, the result, I think, of inoculation.

Waterport. F. C. Broadwell.—Continuous rains prevented seeding in spring as expected. Catch remarkable and growth fine for the time sown (August 22). Benefit of inoculation very noticeable.

Willard. Frank L. Warne, steward, Willard State Hospital.—Beneficial and satisfactory. A portion not inoculated does not show the sturdy and healthy growth that the main portion of the field does. Two crops have been cut, August 10 and September 30, though not seeded until June 16.

Youngstown. Elbert L. Baker.—Nodules seem abundant, and color of plants good throughout the season.

North Carolina, *West Raleigh.* C. K. McClelland.—Four cuttings have been made; second and third cuttings contained much alfalfa. Examination shows plenty of tubercles on the roots, so inoculation was successful.

Ohio, *Cincinnati.* Jas. P. Holdt.—A good stand, while another field about same quality of ground not inoculated had a poor stand and was severely affected by the drought. Tried seed inoculation and ground inoculation, and there seemed to be no difference in results.

East Springfield. Jos. D. Flenniken.—On examining the plants July 8, every plant had the nodules formed on the roots. I think it a success. So far I am pleased with result. (Later, November 2.)—I have a good stand of alfalfa and it is at present about 10 inches in height. My neighbor planted some same day I did, with the same attention and same treatment, except he did not inoculate. I was in his field October 28, and his plants were small, puny, sickly things and very scarce. What he had I don't think will winter, while

OHIO—Continued.

the roots had no nodules that I could find. My plants have roots as large as a lead pencil and nodules as large as peas on them and as many as fifteen stools on one crown.

Leesburg. Arthur Ladd.—Date of planting May 12; date of clipping August 1. The inoculated seed was 8 inches high and a dark green color. The uninoculated was 2 to 4 inches high and yellow in color. Uninoculated seems to be dying out.

Malta. C. A. Clements.—Got a stand of thrifty growth and of dark-green color. Think the inoculation successful as there are nodules on the roots. Neighbors say if I can grow alfalfa on that land it can be grown anywhere in the country.

Montpelier. D. W. McGill.—Good stand where none stood without inoculation. Am satisfied inoculation helped.

New Alexandria. A. C. Fellows.—Result of inoculation is good. Alfalfa is dark green, while strip not used on is turning yellow.

Sharon. J. B. Keys.—Have a fine prospect; find splendid nodule formations on the roots.

Yellow Springs. M. R. Grinnell.—Seed came up very quickly and has made wonderful growth; roots have nodules on them very thick. Bushel of seed sown on 2 acres with 1 bushel of oats per acre. Harvested 4½ tons of hay at two cuttings.

OKLAHOMA, *Lambert.* T. W. Croxton.—Good, a perfect stand, and of healthy color. On upland prairie.

OREGON, *Applegate.* C. H. Elmore.—Seed all grew and lived through a dry summer on high, dry hill land; bids fair for a good crop next year. Without treatment the seed did not germinate at all.

Bedfield. Albert Mark.—We sowed 10 pounds of seed on about three-fourths of an acre. One-half was richly manured, which did not do very well as it mostly went to weeds. The other half made a good stand, grew 18 inches high, and came up very thick, all without water.

Days Creek. C. N. Wood.—Will say I followed the directions and succeeded well. I planted alfalfa seed May 2, 1904, on fairly good clayey loam. Had to cut it twice. The last cutting one-half ton per acre on August 25, 1904, or in four months from date of planting. I sowed red clover the same day and cut the same date and harvested 1 ton per acre in four months from date of planting. This result is with irrigation. My neighbor sowed alfalfa and red clover the same time I did, also with irrigation, equally as good seed and equally as good or better soil, and his crop did not get large enough to clip at all this year yet, and it looks sickly, while mine is thick and a rich green in color. My crop of alfalfa and red clover is at least 60 per cent ahead of my neighbor's. Mine was inoculated and his was not. I shall use soil from the inoculated field to inoculate other fields of the same kind of crop.

PENNSYLVANIA, *Hookstown.* S. M. Ramsey.—A success at the present time. A good stand and good color. The same ground sowed last year was a total failure; came up sickly and yellow and dwindled away.

Hosensack. E. A. Mackling.—The stand was much thicker than that from seed planted on the same ground the year previous.

Muddycreek Forks. Vallie Hawkins.—Sowed 3 acres without inoculation last year. Good stand but few nodules. Had to resow this year (August 2), and inoculated seed. Roots are well supplied with nodules and I have a good stand, 8 to 12 inches high, on October 18.

Pennsylvania—Continued.

Tyrone. H. C. Blair.—Alfalfa tried last year (1903) did not grow. Inoculated seed produced a good stand. Last measurement of a stock showed plant 12 inches high, root 4 inches. We consider the results fine.

South Carolina, *Williamston.* A. W. Attaway.—Very dry time on it, nevertheless a very good stand. Think inoculation very profitable. Others tried without inoculation and fell behind me.

Tennessee, *Clarksville.* Gold Goodlett.—You sent me a package last year, but the weather turned so cold that I did not sow seed; kept all until spring, treated seed, and secured a wonderful stand; think every seed came up. I mowed it three times.

Columbia. Horace B. Hanson.—It has tubercles formed on the roots; is looking fine and healthy. Some of it is on very thin land. I have been trying this plant on the same land for three years without success.

Texas, *Fort Worth.* W. H. Irwin.—Sowed 1,000 pounds of seed on 50 acres. Obtained one-third more alfalfa hay where inoculated; three-fourths ton per acre first cutting, 1 ton each other two cuttings.

San Antonio. B. G. Barnes.—The inoculated appears to be more vigorous and healthy than that without inoculation, although the latter was planted first and originally came to a better stand by reason of the ground being in good condition at the time of planting, while the inoculated was not.

Vermont, *Randolph.* John W. Burt.—We think the result is very good. If we had cut as a crop this season we would have gotten a good yield, and we are confident that next year will show satisfactory results.

Virginia, *East Leake.* A. K. Leake.—It is 18 inches high and could not be more promising; looks splendidly. You will see by the samples I send you that it is full of nodules, showing in an astonishing manner the bacteria-bearing nodules. There are nodules on every plant I dug up. When I dug up some old plants from a field which has failed, I saw no nodules. No one has ever succeeded with alfalfa here.

Ettrick. W. S. Ivey.—On land sown dry, splendid results; plants 8 inches tall and well spread; on wet land, poor results. Bacteria nodules plentiful on most of plot larger than grains of wheat.

Glenallen. Mrs. Imogen Holladay.—A very good stand. I have been unable to get one without inoculation. The roots are plentifully supplied with nodules.

Norfolk. Dr. Livius Lankford.—A most decided difference between inoculated and uninoculated 4 acres; 4 to 6 inches high (6 weeks old), deep green all over. One acre not inoculated nine-tenths dead, rest yellow.

Occoquan. W. W. Giles.—I sowed the inoculated alfalfa seed May 24, 24 pounds to the acre, with a wheat drill, sowing slaked lime at same time and in direct contact with the seed. It came up splendidly, and, I believe, too thick. Thirty days after it was sowed it was 6 inches high, and is now looking elegant. Twelve years ago I sowed 2 acres of alfalfa here and never discovered a spear of it growing. Of course, this was before the inoculation was known. (See Plate IX.)

Washington, *Belma.* Chas. Richey.—Inoculation very beneficial. Growth had formerly been very poor; plants turned yellow and many died, making it hard to get a good stand. Now difficulty is overcome.

Cheney. Roswell K. Johnson.—My experiment seems to be successful. We have the nodules on the inoculated plants, but none on those not inoculated. I think there is an improvement in the growth of the inoculated plants.

Washington—Continued.

North Yakima. W. J. Hess.—Fourth year. The crop, which had been short, pale, and spindling, took on a darker color after inoculation and made a rank growth. Yielded, I think, about three times as much as formerly; did not weigh.

Sprague. Arthur A. Baldwin.—Results very satisfactory. Yielded about 1 ton per acre on dry hill land. Good prospects for next year. Has had positively no rain since last of May. (Report dated October 9.)

Winona. W. H. Mumford.—Inoculation appeared to be perfect, all plants having good color from the first, and at no time have there been any yellow leaves.

West Virginia, *Berea.* John E. Meredith.—Have been trying to grow alfalfa twelve years, and have now the finest prospect of success that I have yet experienced.

Wisconsin, *Fort Atkinson.* "Hoard's Dairyman," November 11, 1904.—An experimental trial of this method of inoculation was made by Professor Short, one of the editors of this paper, last summer with very evident success. Our field already shows the good effect of inoculation. (The method consisted in going over an alfalfa field which was not thriving with a sprinkling cart containing the culture liquid. The operation was comparatively inexpensive, as a 16-foot pipe drilled full of holes was attached to the rear of the sprinkling cart, the water thus taking a sweep of nearly a rod in width.)

Stevens Point. F. G. Pattee.—Where treated, found some roots with clumps of nodules as large as small hickory nuts; where not treated, only an occasional one.

RED CLOVER.

California, *Arcata.* William W. Turner.—A part of the ground was a loose sand, a deposit from the river. It was a hard matter to get anything to grow on it. Here is where my inoculated clover seed seems to grow and flourish. The rest of the ground was a sediment loam and very rich. It was not long before the pigweed started, and it came so thick that it choked out the clover, except what was on the sand. That is growing nicely; has a nice dark-green color.

Connecticut, *Bethel.* George H. Pearson.—Clover made strong growth before rye was ripe. Cut one ton of red clover the middle of September, after rye was cut. Poor sand and gravelly knolls did nearly as well.

Wolcott. Samuel Wilson.—I sowed about 8 pounds of seed, not inoculated, all over field and 3 pounds of inoculated seed in the form of a cross. Result, cross distinct with clover; balance of field none. In company with Mr. E. R. Bennett, of Storrs Agricultural College, I went over fields about September 1 and found stand of clover apparently increased since harvesting.

Delaware, *Townsend.* J. H. Lamb.—Sown on ground where clover failed in spring of 1903. Now have a beautiful stand all over the field.

Idaho, *Cœur d'Alene.* James Reid.—Considerable improvement. On examination, found few nodules on clover uninoculated and very abundant on clover inoculated.

Dupont. J. H. Coon, sr.—Seed was sown on a plot 6 by 7 feet, and has made a good stand about 10 inches high. I sowed a similar plot with same seed not inoculated, and can not find a single plant on it.

Illinois, *Anna.* J. W. Fuller.—Splendid. Got good crop where I had failed eight years in succession.

Emington. C. H. Gilbert.—A more vigorous growth than where seeds were not treated. Made a good growth where I could not raise clover in former trials.

ILLINOIS—Continued.

Neoga. C. L. Wallace.—Seed treated with bacteria was seeded on extra thin land and secured very good stand.

Rantoul. Karl Ekblaw.—As the clover was plowed up, no accurate estimate of benefit could be calculated, but the stand of clover was at least 25 per cent better upon inoculated ground.

Winslow. J. H. Benfer.—Used inoculating material and the clover roots have the little nodules on them all right. Other fields, not inoculated, have no signs of nodules.

INDIANA, *Colfax.* T. C. Holloway.—The clover was pastured after the crop of wheat was taken off. Can give no exact figures. It was sown on white-clay land that has been producing very poorly. It now seems equally as good as that on the black land.

IOWA, *Muscatine.* Charles A. Price.—Clover sowed with oats. The oats showed an increase of 15 bushels per acre over oats on same ground where no treatment was given. An examination of the clover roots showed 75 per cent more nodule formation than on that from untreated seed.

Shellrock. John McNamara.—Good. The land was worn out that the clover was sown on, and clover would not grow there without the inoculating material. I have tried clover on the same ground for the last four years and it would not grow.

KANSAS, *Burlington.* John W. Alexander.—Plots 1 and 2, 4 acres each, yielded three-fourths to 1 ton per acre; seeded April 15. Plots 3 and 4, 1 acre each, not inoculated, but seeded at same rate and at same time, very weak; not much growth, no hay cut.

Kansas City. John Porty.—The clover on 5 acres was about 35 per cent better where the seed was inoculated than the other 5 acres where the seed was not inoculated.

Winchester. B. G. Jeffries.—As clover will not do any good in Oklahoma, I was surprised at my success, and think it just the thing for Oklahoma.

KENTUCKY, *Hopkinsville.* Ben C. Moore.—Cut 2 acres of clover which had been inoculated and 2 which had not been, and find that there is a difference of about 500 pounds per acre in favor of inoculated seed.

Olmstead. John T. Young.—I think the clover will live. Good stand at present (October 26), although we have had the most severe drought since July 15 I ever saw. All other clover sowed at the same time is dead.

Warsaw. E. A. Rea.—Have a good stand, with a prospect of a fine crop next spring. Small plot in middle of field not inoculated all died out.

MAINE, *Augusta.* John Jackman.—The very best results. I soaked or moistened seed carefully, as per directions, and reserved small piece of ground for test; rest of ground was sown to same kind of seed, but catch on inoculated patch is noticeably stronger. It seems as if every seed came up and grew.

Portland. W. S. McGeoch.—Increased yield about 20 per cent.

Wayne. S. H. J. Berry.—Have in previous years had very unsatisfactory results in getting a catch of grass, and especially clover. I tried the bacteria for this crop and am well pleased with the results.

MARYLAND, *Grayton.* Rev. William Brayshaw.—Report on clover sown September, 1903, at Valley Lee, Md. I sowed two lots of seed side by side, one inoculated, the other with 100 pounds of South Carolina rock. Inoculated made double the growth and bade fair to give three times the quantity of hay. (A later report states that the clover was pastured, and no figures as to final yield could be given.)

MARYLAND—Continued.

Smithsburg. S. H. Buhrman.—Set of clover is 50 per cent better at this date than it was one year ago at same date. Never had a finer set of clover. Certainly must give the inoculation the credit for fine stand.

MASSACHUSETTS, *Boston.* Israel Lefavour.—Fully double over that where no inoculating material was used. The increase would have been much larger proportionately on poorer land, I think. I shall try on land next year that has not been fertilized in twenty years.

Concord. Wilfred Wheeler.—The plants were large and very heavy, some growing 3½ feet high. I am satisfied the result was due to inoculation. (Seeded April 20, report August 1; only three months' growth.)

Pittsfield. W. R. Stevens.—Used 8 quarts of seed to the acre with timothy and redtop and have never seen a finer growth of clover. To test the inoculated clover seed on poor soil (or no soil) on a side-hill pasture where to my knowledge it has not been plowed for over 60 years—the soil all washed off and no vegetation growing which stock would eat—I spaded a small piece, sowed clover, laid on brush to protect it from cattle, and the result was a thick, rank growth of clover not only where the ground was spaded but several feet below where heavy rains washed the seed down, thus proving the value and benefit of the microbe inoculation beyond the chance of my doubting.

MICHIGAN, *Fennville.* Chas. E. Bassett.—Inoculated part of field gave 12 per cent more yield, and nodules on roots were as large as small peas, while on that not inoculated the nodules were extremely small.

Manton. Will S. Felton.—The clover on the hills and light spots is fully as good as that on the heavier soil, and the stand is much more even and vigorous than untreated seed on similar soil.

Napoleon. E. L. Griffin.—A glowing success till about June 30, when the crop was killed by excessive drought. One-half acre in protected place a splendid success. On the half acre great numbers of nodules are present, and rank growth of clover.

Royal Oak. H. C. Wilson.—This season has been very unfavorable for clover in this locality. Will say that no one near here has a catch of clover that I know of except myself. Have a good catch of clover on three-fourths of my field of 7 acres. Believe it was because I used clover culture.

MINNESOTA, *Campbell.* N. W. Ware.—Bacteria nodules show in abundance and plants very thrifty. Sown on 15 acres of northwest Minnesota Red River prairie soil with best results. Former owner tried in vain for years to get a stand of clover.

Montevideo. John C. Lucas.—Have an extra good stand, and the finest roots full of nodules of very large size. This clover was grown on land that never had clover on it before.

MISSOURI, *Cabool.* C. L. Morris.—Sowed two plots. Plot 1 was inoculated and has made a fine growth. Plot 2, not inoculated, has nearly all died out. Plot 1 a success; plot 2 a failure.

Chapel. Verona Jones.—I inoculated the soil and I harvested two crops off the ground that was treated. The last crop was well filled with seed. I believe inoculation to be a great help here, as far as I have tried it.

St. Louis. G. S. Myers.—One-third better in growth and appearance than the uninoculated.

Sedalia. Jay H. Decker.—Where seed was treated the stand was nearly twice as heavy. The ground was sown with same seeder, adjusted the same.

NEBRASKA, *Elgin.* Marcus Brown.—Inoculation successful, nodules appearing quite plentiful, though uneven. Crop appears best I ever saw in Nebraska.

NORTH CAROLINA, *Loftis.* Benj. G. Estes.—I have a fine catch of clover where I have not been able to get clover at all. In fact, the farmers say clover will not grow here at all.

Skyland. A. B. Case.—Find at least a gain of one-third more nodule formation over the seed not treated. A success according to my investigation.

NEW YORK, *Adams Basin.* C. O. Barclay.—I never had as good success with clover seed before. It looks as if every seed germinated. I have failed for the past three years.

Albion. Oliver A. Paine.—We had good success with our clover. One-half larger where we used inoculating material.

Butterfly. J. E. Baker.—I have a good stand of clover on ground that I did not expect could grow it successfully. It came up the soonest and rankest in the spring of any I ever grew.

Ghent. George T. Powell.—Sown in orchard for cover crop. On October 15 the inoculated seed stood 4 inches higher than adjoining untreated, while nodule development was greater. The gain is more marked on this poor land than on fertile.

Holland Patent. H. W. Dunlap.—My tenant reports the best stand he has had during his occupancy of the farm, and that upon a hillside where until then he had never been able to make red clover grow. Plants I examined in August showed nodules in every case. Having more of the culture liquid than could be used upon the seed, I distributed this on some light loam, which, after stirring and drying, was broadcasted upon a small part of a field already in clover. My tenant reports that the color and size of the clover indicated the distribution of the soil perfectly.

Prattsburg. B. I. Graves.—The clover where clover inoculation was used was far better than where sowed without. A perfect success. The inoculated ground the poorest; sowed as other seed.

OHIO, *Cincinnati.* C. M. Anthony.—We inoculated our seed, and our clover has made most excellent growth on very poor sandy soil in the fruit district of Michigan.

Seaman. Ira C. Howard.—A fine set on clay upland. I sprinkled the water that was left after soaking the seed over the ground in spots; every spot is plainly visible.

Sidney. Miss Ida K. Wilson.—Perceptible nodules, though not much larger than pin points, but both root and top development much greater than that produced from noninoculated, and the latter produced no nodules. Soil a worn-out clay.

OREGON, *Corvallis.* C. A. Bareinger.—Inoculation apparently good. Plants vigorous. Best stand I ever had—more favorable seasons not excepted. Attribute some of growth to land plaster. I believe the inoculation a success.

Creswell. R. D. Hawley.—Made a fine growth, 12 inches high, and thick. Another piece sowed at the same time on an adjoining field died out. Nearly all the inoculated is all right and a success.

PENNSYLVANIA, *Arthurs.* J. E. Breniman.—The growing clover is thrifty and well rooted. It will do well on poor Pennsylvania soil.

Center Hall. John F. Alexander.—The result of inoculation has been very satisfactory, having fully twice the growth as compared to seed sown sooner without inoculation. I am favorably impressed with this system of fertilizing.

PENNSYLVANIA—Continued.

Freeport. George T. Ralston.—Have secured a good stand of clover on an old worn-out field that I had failed to get clover on three times in succession. Regard the treatment as a success.

Southport. F. L. Bray.—Clover looks fine in lot where inoculation was used; scarcely any in lot where no inoculation was used. Both lots with same soil, same methods of cultivation, same nurse crops, and same time of sowing.

TENNESSEE, *Gruelti.* Ig. Schlageter.—Inoculation satisfactory, twice as much as on the side not inoculated. Did not harvest it this the first year, but it was twice as large as the uninoculated.

VERMONT, *Berlin.* William McCarthy.—Appears to be good; clover is better where inoculated than any other place in the field.

VIRGINIA, *Flatridge.* J. W. Perkins.—The clover is two or three times larger than portion of field not treated. Can tell where inoculated as far as you can see the field.

Meadows of Dan. Ira J. McGrady.—Inoculation good; the roots of plants are covered with nitrogen traps. Some plants contain as many as 100 nodules to the plant. I have a good stand on land that had been cropped twenty years and never sown to grass.

Sandidges. W. S. Gill.—Seed inoculated produced clover 18 to 20 inches high at this time and blooming. That not inoculated 6 to 8 inches high and sickly looking; not blooming. I have all confidence in the bug and believe it will restore clover to us again.

Wolftrap. E. W. Armistead.—Put the bacteria on 2 acres and then sowed the seed. I got the finest stand I ever saw and a foot high by July 1. (Sowed in March.)

WASHINGTON, *Bothell.* Harry G. Brower.—Mixed the material according to directions and thoroughly wet 10 pounds of red clover seed three times and dried each time. What liquid I had left I mixed with 25 pounds of dry dirt and sowed this on 1 acre; harrowed three times. Season was very dry, but the seed lived through and the ground has a good stand. In fact, I am the only one who has a good stand. People told me the soil was too poor for anything.

Marcus. I. T. Peterson.—More than doubled the yield of the clover. Seed was all sown on a uniform soil, and at the same time, side by side, inoculated and not inoculated.

WEST VIRGINIA, *Elkins.* John B. White.—Inoculation good. Moistened 6 gallons of clover seed with the inoculating material, sowed with oats on 5 acres. Nodule formation on clover roots very good. Very good stand of clover on ground. Have sowed clover on same ground previously with poor results.

WISCONSIN, *Iron River.* Joseph Yerden.—I had sowed clover on same land two years in succession and could not get a catch. I used the inoculating bacteria that you sent me and have a fine stand of clover.

NOVA SCOTIA, *Halifax.* Arthur P. Silver.—The clover has grown remarkably strong. The roots are full of little white nodules, which appear to be absent in roots dug up in other parts of farm. Soil was a run-out pasture.

COWPEAS.

ALABAMA, *Fruitdale.* George W. Dibble.—For two years previous to this year I had sown this land to cowpeas. The stand each year stood about 12 inches high. I was not able to find any nodules on the roots. This year with the inoculated seed on the same ground the peas were about 3 feet high, and the roots were covered with nodules from the size of a pin head to that of a pea. I am well pleased with the result.

Muscadine. C. H. Koentz.—Healthier growth of vines and increase of seed pods of from 15 to 25 per cent. The effect of inoculation was plainly visible on poor soil, but on more fertile soil the difference was very slight.

Pineapple. F. I. Walthall.—Increased crop one-third, notwithstanding protracted drought and lack of proper cultivation.

Stockdale. J. L. Stockdale.—Increase of yield about 200 per cent. A very good crop of pea-vine hay.

FLORIDA, *Avonpark.* John Lancashire.—Result of inoculation good. Ground thickly covered with running vines from 10 to 12 feet long. Did not harvest; want to improve soil. Could not get any crop for two years previous.

De Land. A. Cosner.—Inoculation good. Cut for hay; produced double the amount of those not inoculated.

Hanson. O. C. Gramling.—Peas came up; two-thirds stand made moderate vine and leaf and fruited fairly well. Drought cut the crop one-half, though I gathered at the rate of 5 bushels per acre on land that would not yield one bushel per acre last year.

Jacksonville. Millard F. Webster.—A strong bush and heavy yield of peas on part of field inoculated; not enough peas to pay to harvest on part of field not treated. Have planted peas on same land twice before; each time a very small crop.

Pensacola. Geo. W. Howes.—Result of inoculation good. I planted as a fertilizer on poor sandy black-jack land and got a third better results without manure, but inoculated, than on the same land with cotton-seed meal as a fertilizer.

West Palmbeach. G. W. Idner.—On part of land more than 100 per cent difference; on other dry land, 50 per cent. There is no doubt but it will pay all planters to use the culture.

GEORGIA, *Athens.* H. B. Mitchell.—Increase of seed fourfold. Vines were small, owing to severe drought extending from middle of August to November 2. It paid well. Will want more next spring.

Bluffton. P. H. Thompson.—Inoculation of peas with bacteria sent to me, and others at my request, was quite satisfactory. Yield nearly double that of cowpeas planted under same conditions without inoculation.

Columbus. C. B. Gibson.—A fair crop without fertilizer on land that did not grow weeds 10 inches high. Peas not inoculated did nothing.

Covington. John A. Porter.—The peas were planted in drills and on sandy land, and until the drought they showed a marked improvement over adjoining land with 150 pounds acid to the acre. The severe drought in this section has made any other comparison impossible.

Macon. A. F. Jones.—Mixed the culture with dirt and put in the furrow with the seed. The land was very poor and we did not use any other fertilizer. The yield was three or four times as large where the bacteria were used.

Georgia—Continued.

Rome. Hamilton Yancey.—The growth has been rank, of rich dark color over the entire field that was seeded. A difference in favor of the inoculated pea was quite noticeable. My neighbors and friends who have seen the field insist that the field is seeded with a different kind of pea. I wish to express to you my satisfaction and gratification with the experiment. I believe the work you are doing is of inestimable value to the farmers of our country in the future redemption and improvement of our lands.

Stilson. L. P. Garrick.—Planted on rich land, no good was perceptible; planted on rather poor, sandy land, the result was yield trebled.

Stone Mountain. Arthur B. Kellogg.—It is with pleasure that I can report a marvelous growth of the cowpeas which I inoculated with your bacteria last spring. The comparison between the inoculated and uninoculated peas is most pronounced, I should say a difference of 50 per cent.

Illinois, *Mount Vernon.* E. M. Dana.—Sowed in orchard. Each alternate space inoculated shows a great difference in rankness of growth over uninoculated, especially on yellow soil badly worn.

Indiana, *Milan.* James Tribbey.—Cut for hay. Estimated difference between inoculated and uninoculated 300 per cent in amount of vines, hay, etc., in favor of inoculated. No difference in amount of peas.

Willoughby. Louis A. Russell.—A great deal more vigorous growth and healthier vines, with heavier and better stand.

Kansas, *Walnut.* H. C. Coesten.—Inoculation was perfect and satisfactory. Would prefer this method of inoculation to the sowing of soil from field to field; by the latter a person is liable to transfer plant disease. I transplanted the leaf blight to my field a few years ago by doing so.

Kentucky, *Logansport.* R. M. Humphreys.—Two plots same size, same amount of seed sown, with same conditions all round, and the inoculated plot had fine nodules, while the other, that was not inoculated, had but few.

Newton. C. H. Hatchett.—I had a fine yield of pods upon ground that had not grown good crops for many years, and yield of vines was also good.

Winchester. Dr. M. S. Browne.—Estimated weight of hay increased threefold or more; peas fully as much increased.

Louisiana, *Cades.* C. E. Smedes.—Increased the nodules 75 per cent more than peas planted next to them, and vines were more luxuriant.

Lafayette. Ray Fiero.—In 1903 I sowed peas on a side hill and the peas did not grow over 8 inches high, with very small nodules. This year the inoculated peas sown under same conditions made a growth at least four times as great.

St. Martinville. George Lind.—Cowpeas grew well, forming nodules in plenty. I consider the inoculation a success.

Maryland, *Chaptico.* William H. Gardiner.—The 2 acres inoculated grew twice as large, as peas were more prolific than uninoculated part. In fact, the 2 acres were the only part harvested. The rest of the field was insignificant.

Missouri, *Marionville.* U. L. Coleman.—Where inoculation was used the peas did a great deal better and produced fully one-third more. I found few nodules where the inoculation was not used, but where inoculation was used the roots were literally hanging full of nodules, some as large as peas. I showed samples to several of our farmers, and they all stated they had never before seen as many nodules on one vine.

MISSOURI—Continued.

Princeton. Philip C. McDonald, jr.—Produced about as much again hay with the inoculated cowpeas as with those not inoculated. A very large development of nodules on the roots.

NEW JERSEY, *Metuchen.* Frank M. Moore.—Inoculated peas grew rapidly; large leaves and heavy. Uninoculated check rows were decidedly poorer in growth and texture.

NEW YORK, *Stanfordville.* Albert Knapp.—This seed was sown in drills on worn-out land that has been producing very little for a number of years. I had a very fine growth of vines, with plenty of nodules on roots.

NORTH CAROLINA, *Asheville.* Fred Kent.—Inoculation very good. Am only a book farmer; can not give exact figures. Farmers in the neighborhood wish to know how such peas were grown, as theirs were failures.

Lawndale. F. Y. Hicks.—Rank growth 2½ feet high on land that did not make peas before. Well pleased with the result.

OKLAHOMA, *Crescent.* C. B. Fail.—The inoculation was perfect. The crop of peas and hay was about double that that was not treated. Am well pleased with results obtained.

Crescent. A. W. Sanderson.—Seed was planted on level and given only one cultivation. Result was an enormous growth of vines and about 50 per cent increase in grain over same crop and cultivation last year. Think the nodule formation will greatly help soil.

McCloud. Jesse Hearn.—Rapid growth; quick development; 20 per cent increase in yield. Roots full of nodules. Land in fine shape for next crop.

PENNSYLVANIA, *Hartstown.* J. T. Campbell.—Where soil was inoculated the result was marvelous, four times as great as where there was no inoculation. Nodules one-half inch in diameter.

Manheim. S. R. Nissley.—The plot in cowpeas was just double over the plot that had not been treated.

Wilkinsburg. R. G. Atkinson.—Season very unfavorable, yet the inoculated seed came to nearly a perfect stand, more robust and quicker growth. The difference was quite marked. The method is evidently a success.

SOUTH CAROLINA, *Aiken.* Miss Louise P. Ford.—On 1 acre we planted cowpeas broadcast. On one half of this acre we planted one-half bushel of inoculated cowpeas, on the other half acre we planted one-half bushel of uninoculated cowpeas, plowing them both in just the same way. About the middle of June, when harvested, we gathered 1,375 pounds of hay from the inoculated half acre; from the uninoculated half acre we gathered 750 pounds. The land is known as poor sandy soil, and we did not enrich. This is the result of Miss Pellew's and my experiment on Twin Flower farm. (A fuller account of the above experiment appeared in the Aiken (S. C.) Recorder of October 6, 1904.)

Aiken. G. L. Toole.—The land selected for planting was very poor sandy land. The crop has not been harvested yet (October 10). Peas planted on the same land last year failed to make any peas at all. The peas this year, after being treated with bacteria, are very fine. They grew off like they had been highly manured, but were not. The yield will be increased fully 75 per cent. The vines are full of ripe peas. They bore well in spite of the long drought.

SOUTH CAROLINA—Continued.

Albion. J. E. Stevenson.—Yield of peas increased about 100 per cent. Number of tubercles on roots considerably increased.

Columbia. Ralph Osborn.—Sown broadcast for hay. Cut and hauled in 8 tons of cured hay off the 4 acres. The tubercles were plentiful and large, very satisfactory. That sown May 30 made as good hay as quick as that sown earlier. Everyone who saw it said it was the best piece of cowpeas they had seen about here.

Orangeburg. F. M. Rast.—I tried the inoculated by side of stable compost and will say that it was just as good as those fertilized with compost. I am well pleased with results.

Sandyrun. Charles G. Sonntag.—Increase 30 per cent, nodules prominent, vine growth doubled. Planted on sandy loam and clay subsoil, 6 acres; 2 acres not inoculated, no signs of nodules on them.

Sharon. W. T. Feemster.—I harvested twice the amount of peas that I had ever harvested on the same land. I should like to try more of it next year.

Troy. H. B. Blakely.—Inoculated vines weighed three times as much as those treated the same way on same soil not inoculated. Results interested many of our people.

TENNESSEE, *Grandview.* M. L. Abbott.—Owing to circumstances crop was not saved for fodder, but those who were familiar with ground estimated it double, both peas and fodder, that on same ground in former years. Roots loaded with nitrogenous matter as never before.

Ripley. M. M. Lindsay.—Five times as much vine and leaves and two times as much peas as planted on same land without inoculation. There can be absolutely no doubt that above results are due to inoculating seed.

TEXAS, *Bryan.* J. Webb Howell.—Increased yield fully 33⅓ per cent. I believe inoculation is a good thing.

Morales. T. J. Nolen.—A probable increase of 10 per cent over noninoculated seed. Owing to harvesting with swine, I can not be exact about results.

VIRGINIA, *Ashland.* J. P. Wightman.—Seed inoculated very fine; balance of growth small. Nodules very large and in great numbers. Season very unfavorable for peas.

Cedon. Robt. B. Taylor.—I planted the peas in rows 3 feet apart, cultivated them twice, and I found very decided benefit. I left out two long rows without inoculation, and the results could be plainly seen all through the season and also at harvest; a revolution and a revelation.

Danville. T. L. Smith.—The pea vines were the finest I ever saw. I measured some vines 12 to 15 feet long. I made three times as much hay to the same quantity of seed as I ever made. I am pleased.

Ionia. R. Dewsbury.—Had peas on same ground as last year; were more than twice as good and no help given. Last year had no nodules, this year had. Something increased the yield of peas and vines 100 per cent.

Petersburg. Duncan Wright.—Very satisfactory. Am unable to state exact amount of increase; think gain of one-third. Had peas on same land last year when there was no nodule formation on roots; this year on almost all.

Greenbay. Delbert Haase.—Forty-five per cent better in the amount of nodules in test of separate fields. I was well pleased with the result.

Simplicity. Mrs. Rose Fisher.—All the roots simply loaded with nodules. A piece of cowpeas on an adjoining field, not treated, had about one-half as many.

GARDEN PEAS.

CALIFORNIA, *Mesa Grande.* Morgan R. Watkins.—The peas were planted in the poorest soil, scarcely good enough for grapevines, little rain fell, as the year was a dry one. The vines were not more than 1 foot high, yet I gathered an abundance of the sweetest, tenderest pods. Peas not inoculated amounted to nothing. The same results were noted in the black-eyed peas.

Nellie. T. O. Bailey.—Result of inoculation good, 100 per cent better than those not inoculated. Crop was used green.

FLORIDA, *Saint Petersburg.* S. S. Stults.—Most excellent. Compared with what we usually get from same soil and same treatment, I got four times as many peas as we do without the microbes.

ILLINOIS, *Tamaroa.* W. J. Appel.—Owing to the wet weather this spring, could not get seed in as early as I would have liked to do, but result was very satisfactory and increased crop by about 40 per cent over the same ground where bacteria was not used.

Wichert. P. A. Bonvallet.—A complete success; crop about doubled on ground where peas were never planted before.

MAINE, *Lincoln Center.* C. A. Brown.—Crop about double what I got on seed not inoculated. The stuff is worth a good deal for peas on my soil.

Mount Vernon. Thos. S. Hawkins.—The crop of peas was better than usual, but the effect was not so marked as in the case of the beans; the latter was phenomenal.

MASSACHUSETTS, *Boston.* Jesse M. Gore. The pods were larger, fuller, sweeter, and two weeks earlier than peas planted at the same time and under similar conditions with the exception of the inoculation.

Canton. A. L. Mayo.—I can only say that the farmer says the peas treated with the solution overtook in growth the planting three weeks in advance, and both crops were ready for use at the same time.

Florence. Frank H. Graves.—Picked 43 quarts of green peas in the pod. Vines grew from 7 to 9 feet high, and continued in bearing for nearly one month. Very successful. Remarkable growth of vines and heavy crop of pods.

Sandwich. George H. Credeford.—Planted two double rows about 1 rod long; gathered three times enough for a family of four, in all about 1½ pecks. From same soil and amount of seed gathered only about one-half peck last year without inoculation.

MICHIGAN, *Pellston.* H. L. Millspaugh.—We planted four rows of each seed each way; that is, four using inoculation and four without it, harvesting the peas as a green table crop. The results were very flattering to the use of the inoculating material—fully double yield.

NEW HAMPSHIRE, *Franconia.* L. F. Noble.—There were bacteria bulbs everywhere more than an inch through. It was wonderful and it filled me with hope for the future.

NEW JERSEY, *Chatham.* W. H. Meyer.—Seed germinated somewhat sooner than those uninoculated. I recommend it to anyone to use in place of bone dust.

NEW YORK, *Clay.* Mrs. Arthur Hall.—Entirely successful. Yield wonderful. Culture applied to earth and sprinkled along pea rows. The soil now seems like sandy loam, whereas it was the heaviest of clay before. Celery following peas is very fine.

New York—Continued.

Kingston. Mrs. Clara N. Reed.—Three crops from one set of vines, each crop very full and almost double usual crop in quantity. The inoculation has made worn-out soil very productive.

Northwood. John R. Spears.—The tall vine (3 feet high) was cut from a row that was treated with the culture of nitrogen-gathering germs. This sample fairly represents the growth of all the rows thus treated. The short vine (14 inches high) was cut from the row of vines not treated with the culture. It was the best vine among those untreated. The rows were 4 feet apart and the distance between the two plants was about 7 feet. If you recall that the seed was the Dwarf Alaska, the large vine will seem rather remarkable, I think. The nodules are particularly well worth observing. On July 3, I made the first picking from the plot. On 53 untreated vines, taken as they came, I found 102 pods; on 53 treated vines, taken as they came in the next row, I found 856 pods. The first picking well-nigh stripped the untreated row; the treated vines have yielded two good pickings since, and still another is now filling out. Vines first appeared above the ground on May 17, and they had reached a height of from 2 to 3 inches on June 1. The plot was then of uniform appearance as to the thrift of the vines. On June 1, I watered all the vines in the plot, except one row, with a solution or culture of those germs, made according to accompanying directions, and raked fine dry soil over the ground thus moistened. Since that date all the rows have been cultivated enough to keep the surface soil fine and free of weeds and grass, and all have been treated alike in every particular. No fertilizer of any kind has been applied to any of the rows at any time before or since planting. The quality of the soil is uniform throughout the plot. The soil itself could have had no influence in producing the extraordinary difference in vine growth shown herewith. (See Plate X.) If I seem to be burdening you with details, I must urge as an excuse the extraordinary interest excited by the wonderful success attained by the use of the nitrogen-fixing germs.

Scottsville. Frank Kingsbury.—In light sand, soil very poor. The roots were covered with nodules, the vines a good color, the yield good. The nitrogen-fixing bacteria are certainly a success.

Pennsylvania, *Bryn Athyn.* Mrs. J. A. Wells.—On April 14, I planted three kinds of peas. They came up well, but did not grow rapidly. I had inoculated the seed according to directions. On May 14, a neighbor, having obtained a culture for peas, spared some for me. I inoculated more seed and planted them; then having some of the liquid left, I added water at the rate of one-half pint to 2 gallons of water, and having hoed the soil away a little from the roots of the previous planting of peas (now 4 or 5 inches high), I watered them with the diluted culture and hoed the soil back. Well, now the watered planting of peas is a sight—tall, luxuriant plants covered with fine pods. They are the admiration of the neighborhood. The later planting that was inoculated but not watered with the culture is doing better than any peas I have had heretofore, but not nearly so well as the ones that were watered after they were up.

[*Later report.*]—Four bushels of fine, well-filled pods were gathered. Hitherto our soil would not produce peas to amount to anything. My next-door neighbor has soil exactly similar to ours and manured it more heavily. He used the same seed as I did, but my peas were decidedly finer.

Danville. Mrs. G. P. West.—A good crop where heretofore they barely gave seed; good size, a good growth of vine. It is a great thing for the farmer.

PENNSYLVANIA—Continued.

Erie. J. M. Gordon.—Planted a pint of inoculated peas on April 28 in ground that had not been fertilized. At the same time we planted as much more in ground that had been well manured. The crops from each were about equal. After the vines had been pulled up we planted some string beans in the same ground and are now enjoying the result, the vines being as prolific as if they were the first crop of the season.

Philadelphia. Louis Costa.—Result a good third better than other years with same space planted.

Philadelphia. S. N. Lowry.—Vines yielded once and a half the crop yielded by vines from ground not inoculated but which was manured. The vines from inoculated seed yielded full pods and the peas and beans were larger than those from untreated seed.

Titusville. Geo. L. Benton.—I tried it on peas and it was eminently successful. Where I got no peas last year I had an abundant crop after using your bacteria mixture, and I never had such a crop of peas. I think your discovery very valuable, and I thank you for sending the sample to me.

Westchester. Edw. H. Jacob.—Inoculated peas fully matured by October 1; uninoculated did not flower at all. On September 15, 1904, inoculated peas were 18 inches high, uninoculated 8 inches high. Planting was late, but shows big returns by inoculation. (Date of planting, August 15.)

RHODE ISLAND, *Chepachet.* Henry Parsons.—Should think the result of inoculation to be a benefit amounting to about 50 per cent. Can not give result in figures, as the most of the peas were picked green.

SOUTH CAROLINA, *Gaffney.* Jeremiah Gardner.—My peas were better than the peas of others who used commercial fertilizer, ripened early and evenly, circumstances unfavorable. I consider inoculation a boon to agriculture.

SOUTH DAKOTA, *Lead.* A. L. Read.—Sowed on yellow clay. Had great difficulty to loosen the ground enough to cover the seed. Impossible to cultivate. Harvested about 17 gallons of peas of well-filled pods. On piece of ground same size, seed not inoculated, harvested less than one-half gallon of peas.

WASHINGTON, *Juanita.* W. B. Wittenmeyer.—Planted on unfertilized ground. Vines from 2 to 5 feet high and the crop was at least 100 per cent greater than the same kind of pea planted at the same time not inoculated.

White Salmon. W. O. Cox.—Inoculation a great benefit. Crop about double what it would be without it.

WISCONSIN, *Janesville.* J. T. Fitchett.—Plants were stronger, blossomed two weeks earlier, stood dry weather better, and matured more peas than plants not so treated. In addition, I inoculated seed for four other parties, requesting them to report to me. One man reports 50 per cent better yield. His soil was poor, and the bacteria showed more effectively by contrast. A market gardener reports a larger yield than from similar seed not treated; but to him the best feature was earlier maturity by two weeks. All report favorably, those planting on poor soil reporting the largest increase.

Kewaunee. Thos. Zahorik.—Thrashed from 1 bushel of treated seed 19 bushels. From the other peas I only thrashed 11 bushels.

Marinette. George R. Hawkins.—They were twice as full as those not inoculated. Sandy loam. Most of them used green. The nodules were very plentiful on roots.

WISCONSIN—Continued.

Sister Bay. Adolph Soderburg.—There were more pods on the vines that were treated. About 3 bushels more peas to the acre on those that were treated than those that were not. There were twice as many nodule formations on roots from the treated seed.

BEANS.

ALABAMA, *Fruitdale.* George W. Dibble.—When the crop was ready for market the beans were picked from both plots. The plot that was inoculated kept growing and bearing fruit; on the other plot they dried up. When the beans were gathered the yield on the inoculated plot was more than double that of the other.

CALIFORNIA, *Nellie.* T. O. Bailey.—Result of inoculation good, 75 per cent better than those not inoculated. Inoculated did not mildew, others did.

COLORADO, *Arvada.* A. B. Cole.—Planted 3 acres adjoining 2 acres uninoculated. The inoculated beans produced one-fifth more to the acre than adjoining.

ILLINOIS, *Chicago.* Stuart S. Crippen.—Yield of beans was one-third above average, and product unexcelled in size and flavor for table use. Seed beans are considerably larger than parent beans.

Rossville. I. A. Smothers.—While I did not plant any untreated seed, I can say that the yield was surprisingly great, a matter of remark by everyone seeing them.

KANSAS, *Ford.* R. L. Wilson.—Result of inoculation decidedly good; ground inoculation did a great deal the best.

MASSACHUSETTS, *Boston.* Edw. W. Greene.—The beans seemed to grow a third faster, to be in condition to use some time earlier, and to give me at least one-fourth more beans.

Cambridge. L. D. Evans.—The beans and peas that I put in early in the summer have grown marvelously well, and in soil that did not seem sufficiently fertile to raise anything but tin cans and rubbish.

Middleboro. Fred A. Orcutt.—I planted the same seed and the same amount upon the same ground with the culture that I did without, and the beans that were treated have done much better in every way than those that were not.

North Falmouth. Ella M. Donkin.—The beans were the admiration of all who saw them, and I invited all whom I could interest in them to see them. I had planted in another part of the same garden beans which, although supplied with fertilizer, did not amount to anything, and I decided to try the bacteria organisms, even if it were late in the season for planting. I planted them July 14, and early in September we had fine string beans to use. The pods were large and of excellent quality. They continued to bear until an early frost killed the vines. * * * We examined the roots in different stages and found the nodules well developed.

Pike. H. E. Howard.—Darker green throughout season without fertilizer than uninoculated with fertilizer. Gave as good results as fertilized portion, ripening about as early.

MICHIGAN, *Brinton.* B. B. Stevens.—Plants more vigorous and better podded. Estimated increase of yield not less than 25 per cent. Am well pleased with the experiment.

Saugatuck. F. M. Kreusch.—I gathered the beans about September 20; have only thrashed part of them, but I am sure I will have five times as many as last year on the same ground. I think it is immense.

New Hampshire, *Penacook*. J. M. Masson.—Beans were exceptionally good—at least 20 per cent over last year on same ground. This increase I attribute to the inoculation.

New York, *Jasper*. H. W. Smith.—Those inoculated yielded sixtyfold. Those not inoculated yielded about fiftyfold on the same kind of soil with same care. The beans inoculated are one-third larger.

Kingston. Mrs. Clara N. Reed.—Pods very full of large beans. Some vines had a second crop. The inoculation has greatly enriched the soil, so that it is much better to use for other vegetables.

Penn Yan. John D. Buckley.—The ground was on a side hill, gravelly and sandy, and had been practically worked-out. In spite of this and insect attacks, I had the best piece of beans I ever raised. A farmer living near me planted beans twice in succession on the same land and I helped harvest the beans, but they were hardly worth the labor.

Ohio, *Linden Heights*. E. B. Champion —Beans yielded fully one-half more than untreated. The green beans carried the largest-sized pods I ever saw, but the yield was not increased so enormously as in the case of the wax beans. In this case the increase was so marked as to cause wonder among my neighbors.

Pennsylvania, *Cresson*. V. P. Sanker.—On ground which never before would raise a crop of beans had marvelous crop this year, the heaviest ever raised in this locality. Planted seven rows in middle of field without inoculating, and the old conditions prevailed.

Lockhaven. George P. Singer.—I used them in my botany and nature-study classes in this way. I furnished each student with a number of pots of fine white sand. The same day they planted beans and clover, and also the same kind of seeds inoculated with the bacteria. Each pot was exposed to the same conditions and the inoculated compared as to growth with the uninoculated. There was no especial difference in germination, but when the plants had put forth their first leaves the ones inoculated began to grow much faster than their neighbors. It was not long until they were twice as high, and while the ordinary seeds produced plants stunted and ill-nourished, the inoculated seeds in many cases produced a large bean stalk with fully developed pods and beans. The clover seed showed the same result. Root nodules were formed in great abundance. All in all, it was the most interesting experiment I have ever tried in my classes, and it aroused a great deal of interest in the students. I am confident that if clover and beans will grow as they did for us in sand which was quite free from organic matter, your nitrogen-fixing bacteria will solve many problems for the intelligent farmer.

Northeast. John Wheeler.—Result of inoculation splendid. Refugee beans for canning factory. One-third acre yielded $50 to $60 clear profit. I think it can not be beat by use of fertilizer.

Rhode Island, *Kingston*. H. J. Wheeler, director, Rhode Island Agricultural Experiment Station.—Concerning the wax beans and green-podded bush beans, both are continuing to show very striking benefits from the use of the inoculating material, so much so that I think it would be a very important matter, economically, if one were growing them on a large scale, whether the land was inoculated or not.

Tennessee, *Jefferson City*. J. Porter Corbett.—Difference from one-fourth to one-half in favor of inoculation.

Vermont, *Middlebury*. J. E. Sperry.—Gain from inoculation 11 bushels per acre over seed not treated, planted side by side. There is no doubt but that it is a great help.

WASHINGTON, *Juanita*. W. B. Wittenmeyer.—Inoculated beans at least 100 per cent better than uninoculated on same soil. Very sandy soil and quite dry this last season.

WISCONSIN, *Marinette*. George R. Hawkins.—Produced fully 100 per cent above those not inoculated. Clean and well-formed; no rust.

SOY BEANS.

ALABAMA, *Rash*. W. W. Lee.—All inoculated but six rows. Inoculated began showing result of inoculation in a few days after they came up, and harvested 50 per cent more than the other.

GEORGIA, *Gainesville*. John E. Miller.—The soy-bean inoculation I got last spring was a complete success. I planted 10 or 12 acres on an old barren field, and they are from 12 to 36 inches high, and have not found a single one that was not inoculated, one with tubercles 26 inches from the base. I think your Department a great help to the farmers.

HAWAII, *Napoopoo*. Gordon Glore.—Inoculation successful. Increased growth of plant and abundance of root nodules.

KENTUCKY, *Winchester*. Dr. M. S. Browne.—Twelve thousand five hundred pounds dried hay, ready for storing, per acre; ground where seeds were not inoculated at rate of 1,500 pounds cured hay per acre. Soil, medium bluegrass sod. Noninoculated a failure; inoculated, wonderful crop. Date of planting, April 15; date of harvesting, July 25.

MARYLAND, *Bynum*. Wilmer P. Hoopes.—Our soy beans, drilled in with corn in rows 3½ feet apart, the whole crop making about 20 tons of silage per acre. The beans just covered the space between rows and yielded at least 2 tons per acre. The roots were just covered with nodules.

Smithsburg. C. M. Leiter.—Growth strong, possibly one-fourth more vine than where not inoculated.

MISSOURI, *Marionville*. U. L. Coleman.—Where inoculation was used the beans did a great deal better and produced full one-third more beans. I found no nodules on the soy beans where not inoculated. The inoculation was a success.

NORTH CAROLINA, *Dome*. D. L. Clements.—The lot of land (1 acre) inoculated doubled in yield of hay the lot not inoculated, side by side in the same field on the same kind of land. The growth where inoculated was very luxuriant.

PENNSYLVANIA, *Guys Mills*. William Miller.—The soy beans made twice the growth of former trials. I think the inoculating made the increased growth.

TENNESSEE, *Spring City*. J. M. Thompson.—Large tubercles covered all the roots, crop of stalks and leaves very heavy gain, 10 bushels per acre. Land poor. I consider the inoculation a perfect success.

VIRGINIA, *Carysbrook*. C. E. Jones.—All of the inoculated hills showed an abundance of nodules, while only a total of four were found on the uninoculated ones, notwithstanding the proximity of the inoculated seed, the roots of both plants often interlacing. One row inoculated by culture and one by soil from soy roots having numerous nodules showed an equal number of nodules; the check had none. I find that the roots show far more nodules than I have ever seen before, and this development seems more excessive on the poorer parts of the field.

Gloucester. R. M. Janney.—I did not weigh the hay, but could see a great improvement over the uninoculated seed from the start, and got double the crop in the harvesting.

Greenbay. Delbert Haase.—Thirty per cent increase in nodules over that which was not so treated by actual test of two fields sown the same week. The crop of the treated was better in color and yield on thin land.

VIRGINIA—Continued.

Ingersoll. Charles C. Deissner.—Although there was only about one-third of a stand on account of poor seed, it made five big loads of elegant hay. The plants were over 3 feet high and loaded with beans. I tried 1 acre without bacteria. They were not near as good, either in growth or in yield of beans.

Ionia. R. Dewsbury.—Had the finest crop of beans I ever raised; rather poor ground without anything to help to enrich. I think the inoculation helped wonderfully. Nodules large.

Simplicity. Henry Fisher.—About 80 per cent of the plants contained from 1 to 20 nodules, almost invariably on the main root. No soy beans had ever been planted on this land before.

Simplicity. Mrs. Rose Fisher.—Nearly all plants had from 1 to 29 large nodules, nearly all located on the taproot about 1 to 2 inches in the ground. An adjoining field, not treated, showed but very few nodules.

HAIRY VETCH.

ALABAMA, *Tuskegee.* George W. Carver, director, Alabama Agricultural Experiment Station.—The inoculated plot grew vigorously—in fact, made an enormous growth—and made 7 bushels of seed to the acre. The other was so small that I did not thrash it out.

KENTUCKY, *Trenton.* Phil. E. Bacon.—Used vetch material with best results. The growth was very heavy and the roots as full of nodules as any illustration I have ever seen, some clusters fully as large as the end of my finger.

MISSISSIPPI, *Aberdeen.* Isaac H. Hunt.—Inoculated was better in every way than the untreated seed. We are very much encouraged by what we have already seeded.

MISSOURI, *Rolla.* J. A. Foden.—We have a splendid stand. Color good, and altogether very pleased with result.

NEBRASKA, *Taylor.* Ray G. Hulburt.—Bloomed three weeks earlier; more seed; larger plants. Oats sowed with it were larger. Roots crowded with tubercles, single and in masses. Sowed too close; germs spread to untreated part in July, but it never caught up. Some plants 10½ feet long.

NEVADA, *Skelton.* Jas. H. Campbell.—Best I have ever had. Vetch was so thick I could not see where the machine cut.

NORTH CAROLINA, *Statesville.* F. T. Meacham.—This vetch grew to a height of 6 feet in some places, although, not having sufficient grain crop to hold it up, it fell over very badly. I think the vetch inoculation a great success.

NEW YORK, *Butterfly.* J. E. Baker.—Fine growth on very poor soil. A high, dry, gravelly knoll grew 6 to 8 feet and a mass of blossoms and pods. Have never succeeded in growing anything on this piece before.

OHIO, *Celina.* D. Hellworth.—Nodules are very abundant; simply wonderful. I measured a stalk to-day 7 feet long, besides having four branches.

WASHINGTON, *Seattle.* David B. Porter.—Last fall I treated winter vetches with the solution prepared as directed and planted the same broadcast over a small patch of ground with a good deal of clay, some blue and some shot clay. On turning the ground over in the spring, there was a network of roots forming a thick sod about 8 or 10 inches deep and very heavily charged with the nitrogen nodules, some roots having as many as 40 or 50. I have used other rotted vegetable matter with this to form a humus and have now a fine friable soil which yielded very heavily this year.

WISCONSIN, *Germania*. C. E. Pierce.—The benefit was very plain, promoting a rank growth, adding at least one-third to crop.

Meadow Valley. C. H. Johnson.—Inoculation successful. Nodules in quite large clusters on lower fibers of the roots, more scattering near the surface. Planted on high sandy land.

CRIMSON CLOVER.

ALABAMA, *Tuskegee*. George W. Carver, director, Alabama Agricultural Experiment Station.—This was quite noticeable, that on the adjoining plot the stand was just as good as on the inoculated plot, but it grew very poorly. It remained small and yellow throughout the season. The inoculated plot grew fairly well and was very rank and green in color. These plots were treated in every way alike except in the matter of inoculation. One end of the inoculated plot did not get any of the inoculating material and the small inferior clover was very noticeable.

PENNSYLVANIA, *Bellefonte*. James A. B. Miller.—Fair catch on thin soil. About 6 inches high. Failure on same soil last year without inoculation. Seems thrifty and gives every promise of successful catch.

Joanna. H. E. Plank.—It is a satisfaction to inform you that there was a much greater mass of fibrous roots on the plants grown from the seed treated with the material than on the plants from the untreated seed. The nodule formations are much more abundant on the former class of plants. There is a good stand of clover.

WASHINGTON, *Spokane*. Henry M. Richards.—The results heretofore with the same amount of seed have been a very stunted growth and scant blooming. The seed prepared with the inoculating material has produced a most luxuriant growth and a perfect mass of bloom, an improvement so great that it is difficult to describe.

WEST VIRGINIA, *Elm Grove*. George Fox.—Seed inoculated 50 per cent superior to the seed which was not inoculated.

SWEET PEAS.

CALIFORNIA, *Los Angeles*. W. L. Cleveland.—The seeds were treated in accordance with the instructions you sent me, and then planted in the usual manner. The result of this seeding was a hedge of vines that grew to a height of about 8 to 10 feet, covered with a lot of fine large blossoms that were the delight of the whole neighborhood. Across the street, and treated in the ordinary way with the same seed that I furnished them, but without the inoculation, the vines scarcely grew 5 feet and the flowers were small and few. I consider the thing a success.

MASSACHUSETTS, *West Roxbury*. F. G. Floyd.—Plants were very luxuriant and about 12 feet high. Leaves very large and rotund; flowers very large and of fine color. Plants produced several double flowers, i. e., having two or three entire or partially formed standards.

NEW JERSEY, *Newark*. William J. Hesse.—The crop was a complete success, while other growers in this location did not succeed at all. While I have no record of the quantity of the crop, I will say that I had a larger crop, better blooms of lasting quality than any other grower with the same amount of ground planted. I had two awards at the New Jersey Horticultural Society for these same blooms in June and July at Orange, N. J., and I know that had it not been for the inoculating of the seed I would not have been so successful.

FIELD PEAS.

MAINE, *Auburn.* G. L. Thomas.—The product out of No. 1 strip without any fertilizer was as much as out of No. 3 with the heavy manuring. In other words, the inoculating culture had done as much for strip No. 1 as the barnyard dressing had done for No. 3; while No. 2 (inoculated and manured) had produced as much as the other two strips combined. The growth in No. 2 was excessively strong and luxuriant, and this was due to the nitrogen drawn from the air by the vaccinating cultures. No. 1 was fair yield and cost about 60 per cent as much as No. 2 and about 47 per cent of that for No. 3.

PENNSYLVANIA, *Hartstown.* J. T. Campbell.—Where soil was inoculated the result was marvelous—four times as great as where there was no inoculation. Nodules one-half inch in diameter. (See Plate VII.)

TEXAS, *Keene.* A. P. Wesley.—Nodules formed on vines when quite young and the growth was fine, while the land they were planted on was worn-out clay. I think it a success.

WISCONSIN, *Bay City.* Chicker Brothers.—A very satisfactory crop was raised where failure had attended for seven years.

VELVET BEANS.

FLORIDA, *Jacksonville.* E. H. Armstrong.—Thirty to 50 per cent increase over that where seed was not inoculated with the velvet-bean culture; same for cowpea. Season dry, somewhat unfavorable.

LOUISIANA, *Cades.* C. E. Smedes.—Increased the nodules and the vines 30 per cent. Vines were plowed under.

BERSEEM.

CALIFORNIA, *Berkeley.* David Fairchild.—You will be interested to know that at Berkeley this year there was an immense difference between the plots of berseem from treated and untreated seed, the former being several hundred per cent better than the latter.

PEANUTS.

VIRGINIA, *Poplarmount.* Charles Denney.—Inoculated a piece of land according to your instructions, and planted Spanish peanuts. Increased yield at the rate of 5 bushels per acre.

MISCELLANEOUS.

NORTH CAROLINA, *Gibson.* Dr. N. M. McLean.—As to "nodule formation," a test was made by myself in person to determine this feature. Sterile soil (obtained from a sand subsoil several feet below the surface) to which was added a certain amount of phosphoric acid and potash, obtained from acidulated rock and muriate potash, was placed in one-half gallon pots. Each legume tested was planted in a number of these pots. To a certain number a small quantity of the "inoculating material" was added, with others as "control pots." In each test a marked contrast was noticeable in a short time, the inoculated pots showing several times the plant growth the control or uninoculated pots did, and in each case the inoculated pots showed a plentiful supply of nodules on the plant roots. An experiment on a large scale was then tried. A trench 3 feet broad and 12 feet long was dug out 30 inches deep. This was in a heavy clay-loam soil. The trench was filled with this same sterile soil used in the

North Carolina—Continued.

pots fertilized with the phosphoric acid and potash. In each square (3 feet by 3 feet) a legume was planted—alfalfa, crimson clover, soy beans, and velvet beans. Each variety of seed was inoculated with material you so kindly furnished, and in each test there was an abundant "nodule formation." In each one of these several tests the control pots and plots verified the results beyond the possibility of doubt. I hope next season to be in a position to make a tabulated report that may be of use to others. As to myself, I consider your discovery the greatest one of the age and hope you may live to see a universal acknowledgment of the same.

SUMMARY.

Owing to the direct effect of the nodule-forming bacteria upon legumes, these plants are supplied with a source of nitrogen not available to most other plants. Consequently, the legumes can flourish in a soil practically devoid of nitrogen.

The effect of legumes upon succeeding crops of any kind is generally beneficial, because of the fact that the soil is enriched rather than impoverished by these plants.

Where nitrogen-fixing bacteria are lacking, it is possible to introduce them artificially either by transferring soil from an old field where the desired leguminous crop has been successfully grown, or by the use of pure cultures of the proper organism.

The method of transferring soil is objectionable because of the inconvenience and expense, and is apt to be dangerous on account of the possible transfer of weeds, insect pests, and plant diseases.

The use of the German preparation, nitragin, has not been a success, probably owing to the method of growing the bacteria, which reduced their virulence, as well as their being distributed in a form which caused them to deteriorate rapidly and die.

The nodule-forming organism is a true micro-organism, having three well-defined stages consisting (1) of minute motile rods which produce the infection and frequently form zooglœa masses; (2) larger rods either motile or nonmotile; and (3) capsulated forms, the so-called "branched organisms," which are made up of two or more rods held together in a sheath.

There is but one species of legume organism, *Pseudomonas radicicola* (Beyerinck) Moore. The difference in the infective power of bacteria from different hosts is due to slight physiological variations which can be broken down readily by cultivation.

In order to increase or maintain the virulence of nodule-forming organisms they must be cultivated upon nitrogen-free media. Growth upon rich nitrogenous media tends to diminish and frequently destroys the nitrogen-fixing power, since this element can be obtained more easily from the medium than from the air.

Various external conditions, such as heat, moisture, alkalinity, amount of nitrogen in soil, etc., all have a direct effect upon the

legume bacteria, and the failure of nodules to develop may often be traced to such a cause.

The nitrogen is fixed by the bacteria in the nodule and becomes available by the action of the plant in dissolving and absorbing the combined nitrogen in these organisms.

Nodules inhabited by rod forms of bacteria which can not be dissolved by the plant are of no more benefit than any parasitic gall would be.

There is no true symbiosis between the bacteria and the host. The nature of the nodule-forming organism is purely parasitic, and unless the plant can overcome its action causes distinct harm.

It is possible for nitrogen-fixing bacteria to penetrate the roots of plants and be of decided benefit without the formation of nodules or any external evidence of their presence.

While it is desirable that artificial inoculation be made at the time of planting, experience has shown that under certain conditions crops of three or four years' standing are improved by adding bacteria to the soil.

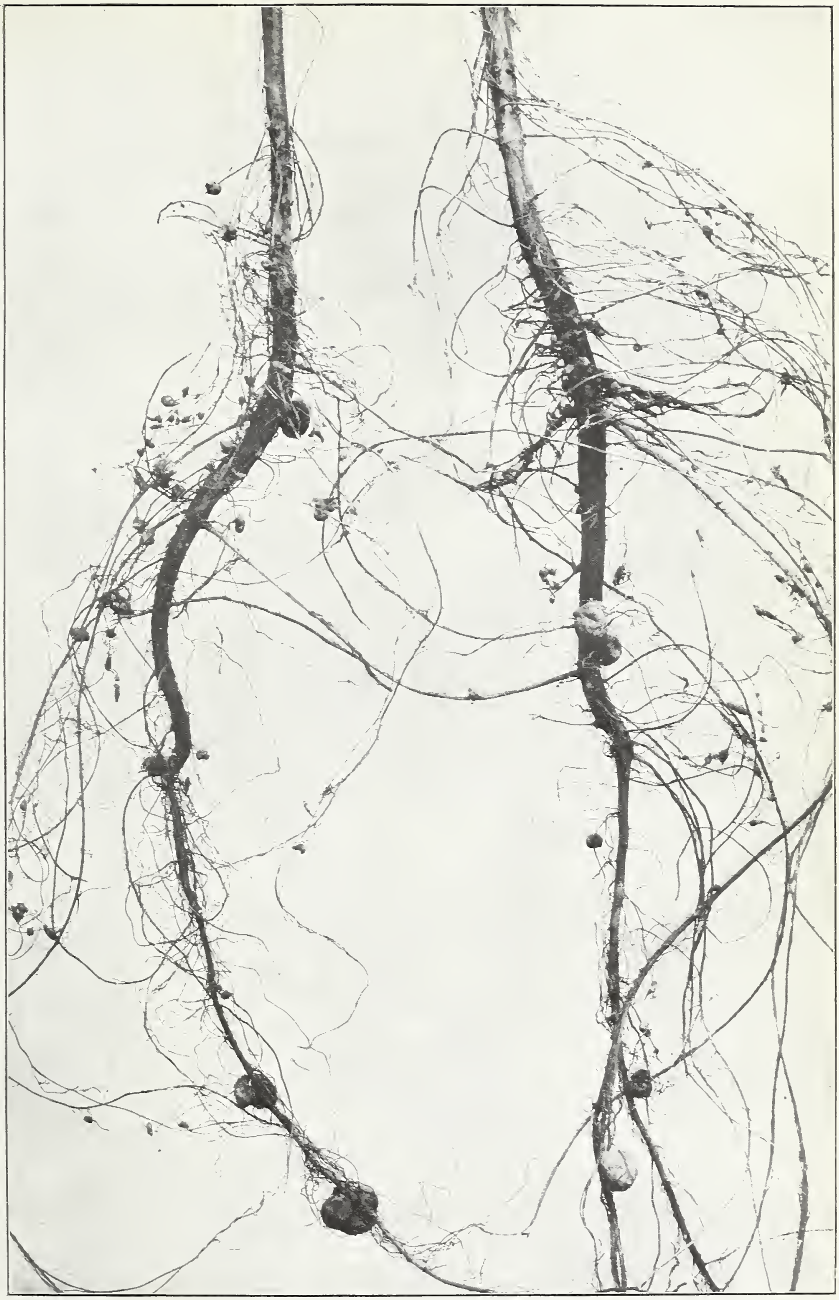
Inoculation is usually of no benefit to soil already containing the proper bacteria, although there may be exceptions. It should not be practiced where the soil is so rich in nitrogen as to prevent the development of the nitrogen-fixing organism.

The inoculation of seed and soil by means of pure cultures grown and distributed according to methods devised by the Department of Agriculture is shown by the reports of practical farmers to be of distinct advantage when used under circumstances that will permit benefit.

O

EFFECT OF RICH NITROGENOUS SOIL UPON FORMATION OF NODULES OF SOY BEANS.

Same culture and seed used as in Plates III and IV.



EFFECT OF POOR SANDY SOIL UPON FORMATION OF NODULES OF SOY BEANS.

Same culture and seed used as in Plates II and IV.

EFFECT OF POOR CLAY SOIL UPON FORMATION OF NODULES OF SOY BEANS.

Same culture and seed used as in Plates II and III.

A.—LARGE ALFALFA PLANTS GROWN ON SANDY UPLAND. INOCULATED AND INTERNAL INFECTION PRODUCED WITHOUT NODULES. *B*.—SMALL ALFALFA PLANTS GROWN ON RICH BOTTOM LAND. INOCULATED FROM MELILOTUS FIELD.

FIG. 1.—ALFALFA ONE YEAR OLD.

Few remaining plants from field which completely failed. Inoculated with culture improperly prepared.

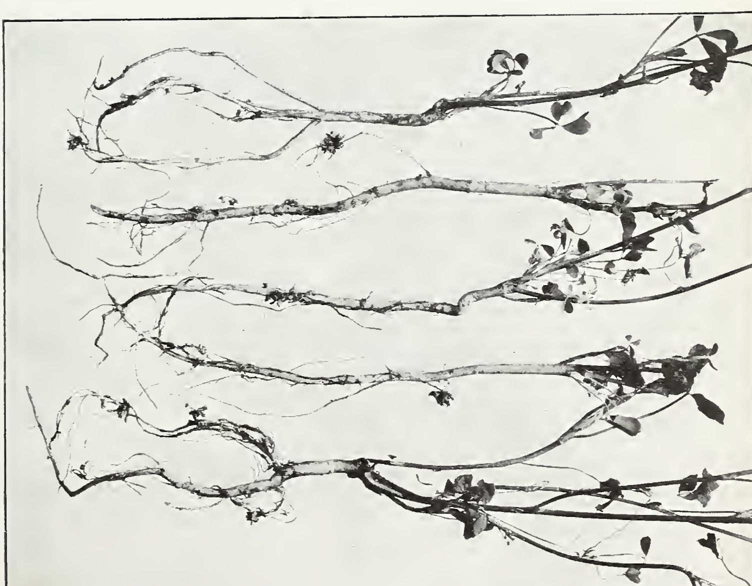
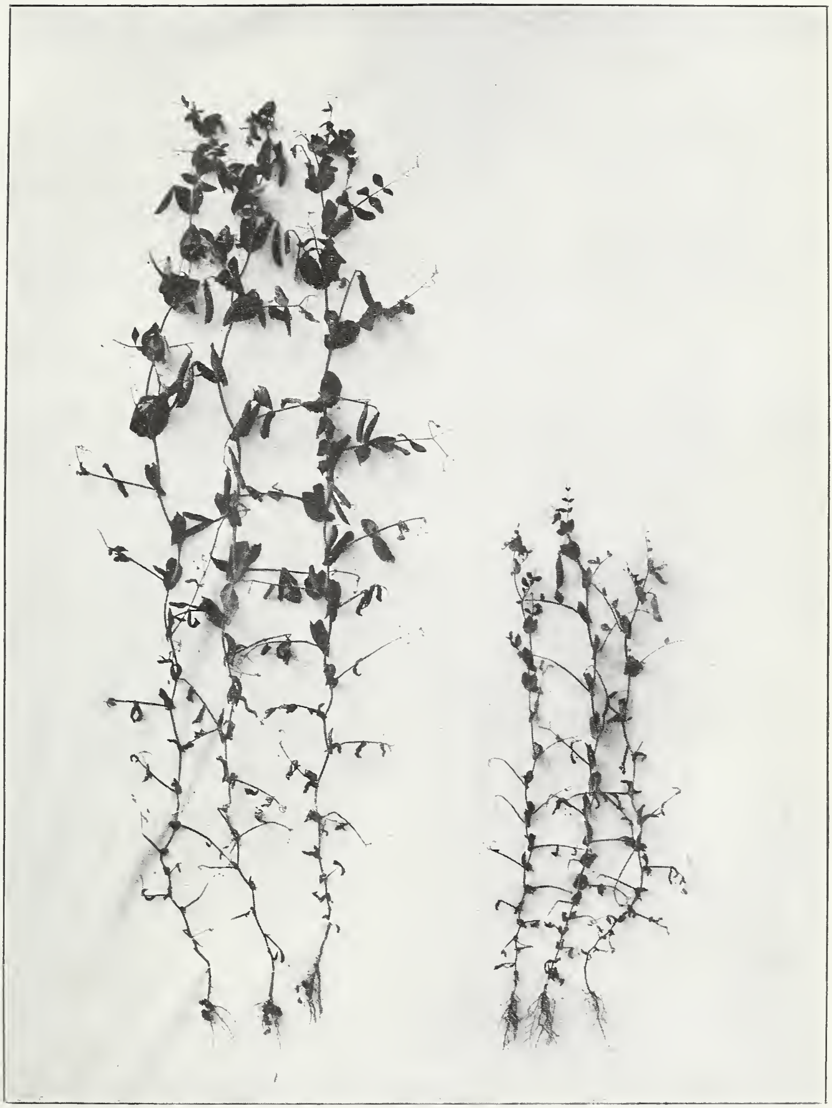


FIG. 2.—ALFALFA TWO MONTHS OLD.

Good stand. Field adjoining one which grew plants in figure 1 and soil exactly the same. Inoculated with bacteria from the same culture used in field which failed, but solution carefully prepared according to directions.



INOCULATED AND UNINOCULATED FIELD PEAS, GROWN BY J T. CAMPBELL, HARTSTOWN, PA.

Photograph by Mr. Campbell.

ALFALFA PLANTS FROM DIFFERENT PARTS OF THE SAME FIELD, THE GREATER GROWTH BEING DUE TO SUCCESSFUL INOCULATION. FARM OF A. W. BRAYTON, MT. MORRIS, ILL.

GROUND THICKLY COVERED WITH ALFALFA TWO MONTHS AFTER SEEDING ON SOIL WHERE PREVIOUS ATTEMPTS TO GROW THIS CROP WITHOUT INOCULATION HAD ABSOLUTELY FAILED TO GET A CATCH. FARM OF W. W. GILES, OCCOQUAN, VA., JULY 28, 1904.

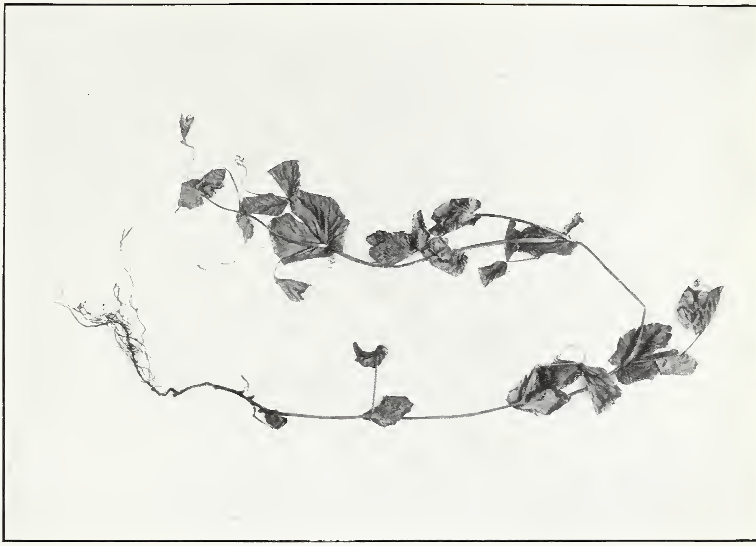


FIG. 1.—BEST VINE IN UNINOCULATED ROW OF PEAS GROWN BY JOHN R. SPEARS, NORTHWOOD, N. Y.

FIG. 2.—BEST VINE IN INOCULATED ROW OF PEAS GROWN BY JOHN R. SPEARS, NORTHWOOD, N. Y.